GROWTH
Genetics & Hormones
Vol. 16 No. 1 March 2000

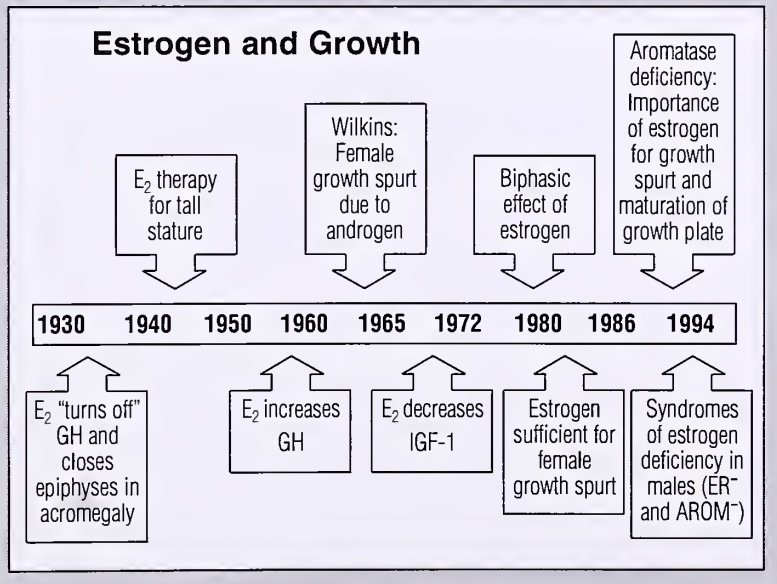
Growth and Estrogen

Graeme R. Frank, MD
Department of Pediatrics
Albert Einstein College of Medicine
New Hyde Park, New York

INTRODUCTION

During the past 5 years we have gained great insight into the critical role that estrogen plays in growth. This article reviews highlights of growth and estrogen research of the past 65 years (Figure 1), points out a number of earlier misconceptions, and culminates in the identification of "experiments of nature" that have revolutionized our understanding of the role that estrogen plays in linear growth.

Figure 1



Time line indicating the highlights in estrogen and growth research over the past 65 years. $AROM^-$, aromatase deficient; E_2, estradiol; ER^-, estrogen receptor deficient; GH, growth hormone; IGF-1, insulin-like growth factor 1.

GROWTH-INHIBITING EFFECTS OF ESTROGEN: THE EARLY YEARS

Children with precocious puberty have short stature as adults as a result of premature epiphyseal closure. Furthermore, in the absence of gonadal steroids, the epiphyses remain open and growth continues. The conclusion from these observations was that gonadal steroids were responsible for closing the epiphyses. Animal studies performed by Zondec in the 1930s revealed that estrogen had growth-inhibiting and growth hormone (GH) antagonistic properties.[1] As a result, it was concluded that estrogen, in addition to closing the epiphyses, "turned off" GH secretion.

As an extension of the assumption that gonadal steroids were responsible for turning off GH secretion, it was assumed that children would have more circulating GH than adults, the notable exception being patients with acromegaly. Beginning in the 1930s, in an attempt to inhibit growth and turn off GH secretion, patients with acromegaly were treated with gonadal extracts[2,3]; then, when pure steroid hormones became available in the 1940s, patients with acromegaly were treated with estrogens and androgens. The results with testosterone were disappointing, but estrogen proved to be highly effective, which was taken as proof that estrogen turned off GH secretion.[4]

In This Issue

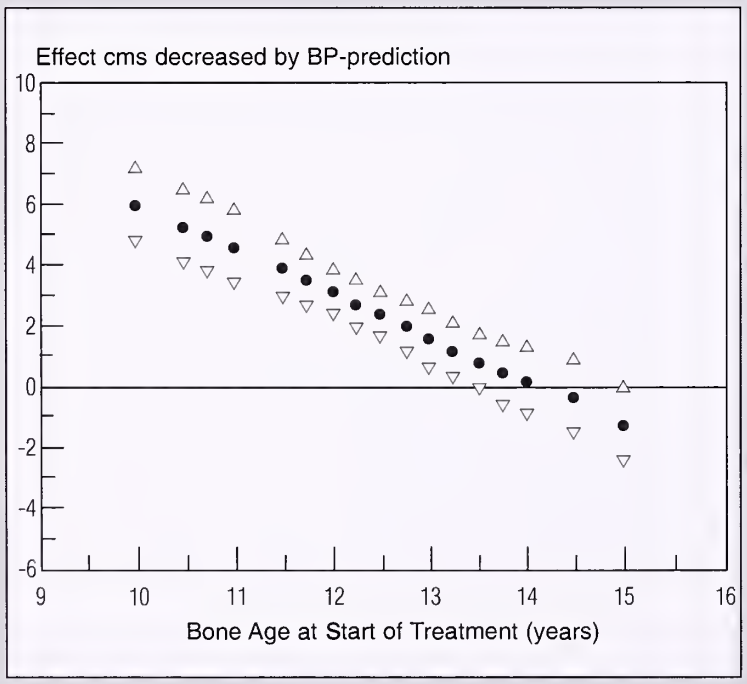
This educational activity is supported by an unrestricted educational grant from Genentech, Inc.

As an extension of the estrogen treatment of acromegalics, the late 1940s and early 1950s saw the start of estrogen treatment of excessive growth in adolescent girls.[5] A number of preparations have been used, including diethyl-stilbestrol, conjugated estrogens, injectable estrogen esters, and ethinyl estradiol. Ethinyl estradiol is the most widely used, and doses have decreased from 500 µg in the 1960s to 200 to 300 µg in the 1970s to 100 µg more recently.[6] Comparing studies is difficult because of the different preparations used, the different doses of estrogen administered, the varied duration of therapy, and the bone age at the start of treatment. Most studies show a growth-inhibiting effect that is inversely correlated with bone age at start of therapy (Figure 2).[6]

ESTROGEN DOES NOT "TURN OFF" GH SECRETION

It was only in the early 1960s that the radioimmunoassay was developed to measure physiologic levels of GH,[7,8] and it was a great surprise that young adults had higher levels of GH than children. Women also were noted to attain higher levels than men. Finally, in 1964 Rabkin and Frantz demonstrated that estrogen increases GH.[9] Therefore, the earlier assumption that gonadal steroids turned off GH secretion was incorrect.

Figure 2



Adjusted effect of estrogen therapy in adolescent females with tall stature. This representative study included 247 girls aged 12.7 ± 1.2 years. There were 88 controls and 159 treated subjects (90% were treated with ethinyl estradiol 200 µg). Duration of therapy was 1.9 ± 0.6 years. Mean length of follow-up was 10.9 years. Solid dots regression line; open triangles represent 95% confidence interval. Adjusted effect of estrogen (cm) = 20.22 – 1.44 × bone age (years).[30]

Reprinted with permission from de Waal WJ, et al.[30]

The immediate question that then arose was, "How, in an individual with open epiphyses, can estrogen slow growth while increasing GH?" The answer to this question came in 1972 from the elegant work of Wiedemann and Schwartz, who demonstrated that in acromegalics estrogen therapy (0.5 to 1.0 mg) caused a rapid fall in insulin-like growth factor 1 (IGF-1) but not GH. IGF-1 rose again when estrogen therapy was stopped.[10] In addition, they demonstrated that in patients with GH deficiency (GHD), estrogen therapy aborted the rise in IGF-1 that follows GH therapy.

Up until this point, only growth-inhibiting actions of estrogen had been demonstrated. In fact, in the Third Edition of Lawson Wilkin's textbook of endocrine disorders, published in 1965, the following statement appears: "Since estrogens have little or no effect upon nitrogen retention and in large doses may even inhibit it, ***it is probable that the adolescent growth spurt in females is due to adrenal androgens rather than to estrogen.***"[11]

GROWTH-PROMOTING EFFECTS OF ESTROGEN

Children with Turner syndrome lack estrogen and also lack a pubertal growth spurt. This observation prompted Ross, in 1983, to study the growth of patients with Turner syndrome in response to different doses of estrogen.[12] She studied 19 girls with Turner syndrome who received estradiol for 4 weeks in doses of 0, 50, 100, 200, 400, and 800 ng/kg/d in a double-blind manner. Patients received up to 3 monthly studies per year, and there was a 3-month washout period between monthly studies. She demonstrated a biphasic effect of estrogen on growth (Figure 3A). At low doses (100 ng/kg/d), there was a marked stimulatory effect on ulnar growth that disappeared at doses of 400 ng/kg/d and higher. This maximal stimulatory dose of estradiol (100 ng/kg/d) has been shown to increase the pulse amplitude of GH without affecting the pulse frequency.[13] However, despite the increase in GH secretion, there is no significant increase in the IGF-1 level at this growth-stimulating low dose.[12,13] Regarding longer duration of therapy, 5 µg ethinyl estradiol therapy daily in Turner syndrome (131 to 192 ng/kg/d) for up to 14 months resulted in increased growth velocity, again with no change in the IGF-1 levels.[14] It is only at the increased doses of estrogen, which have no effect on growth rate, that the IGF-1 levels rise (Figure 3B). It therefore appears that there also is a biphasic response of IGF-1 to estrogen in that intermediate levels of estrogen increase IGF-1 and high doses decrease IGF-1.[14]

THE FEMALE GROWTH SPURT: THE ROLE OF ESTROGEN

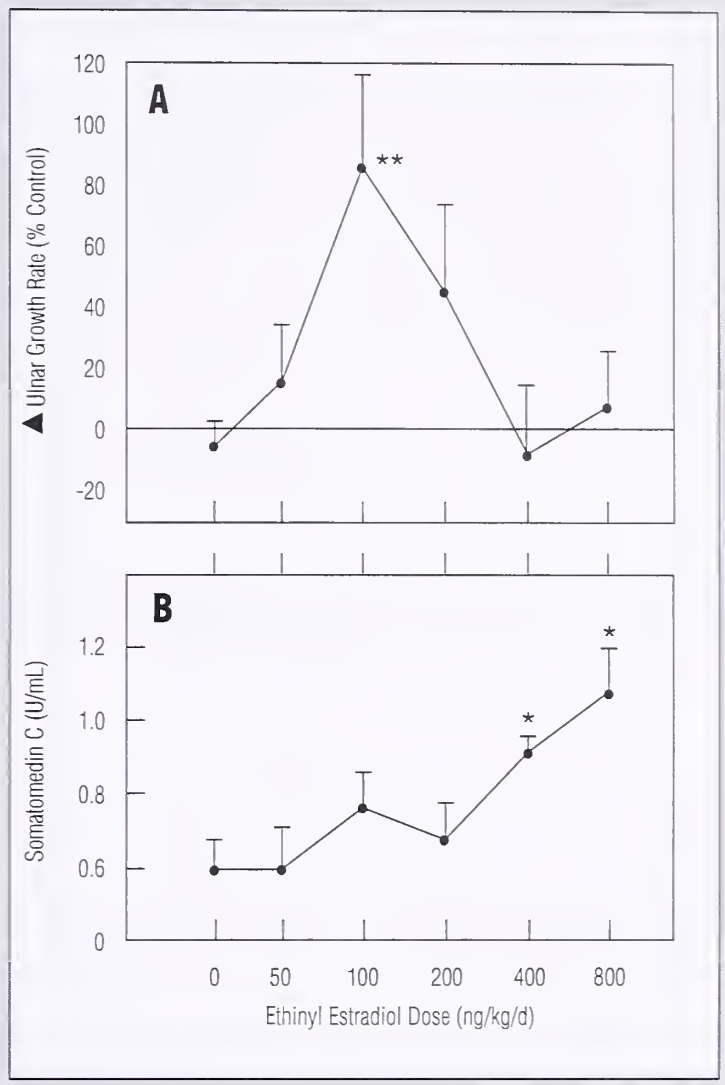
Given that estrogen can stimulate growth in females, it was important to question the assumption that the female growth spurt was due to androgen. Differentiating the roles that estrogen and androgen play in growth is very difficult

since estrogen and androgen are present in each sex. Testosterone is an obligatory intermediate in estradiol biosynthesis and aromatase, the enzyme that catalyzes the conversion of testosterone to estradiol, is found in males as well as females. In order to evaluate the role that estrogen plays in growth, without any influence from androgen, Zachmann (in 1986) studied 8 patients with androgen insensitivity.[15] These were individuals with disruption of their androgen receptors, and therefore they had only 1 functional sex steroid receptor. The pubertal peak height velocity occurred at a mean age of 12.7 years, closer to that of normal girls (12.4 years) than normal boys (13.9 years).[16] Mean peak height velocity was 7.4 cm/y, the same as in normal girls (7.3 cm/y) and lower than that in normal boys (9.3 cm/y). Therefore, estrogen alone, in the absence of androgens, is able to support a normal female pubertal growth spurt, both in magnitude and timing.

CAN ANDROGEN SUPPORT NORMAL PUBERTAL GROWTH IN FEMALES?

The answer to this question came in 1994 when Conte described a female patient with aromatase deficiency.[17] As a result of this deficiency, she had high levels of androgens and low levels of estrogens. At age 14 years, she had no breast development and no menarche. She had Tanner stage IV pubic hair, abundant axillary hair, acne, and an enlarged clitoris. Despite elevated androgens sufficient to produce virilization, she was short (height SDS of –1.5), had no growth spurt and, most remarkably, her bone age was delayed (10 years at chronologic age 14 years). With replacement therapy of 20 µg ethinyl estradiol, there was a striking decrease in her levels of androgens and gonadotropins, and she had a 13-cm pubertal growth spurt. Therefore, the assumption that the female pubertal growth spurt was due to androgens appeared to be incorrect.[11]

Figure 3



Relationship between dose of ethinyl estradiol and ulnar growth rate and serum somatomedin C.[12] *P <0.05; **P <0.025

Reprinted with permission from Ross JL. et al.[12]

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

THE ROLE OF ESTROGEN IN MALES

Since pubertal peak height velocity occurs early in girls and late in boys, at times when estradiol levels are low and quite similar, it has been suggested that low concentrations of estradiol are important for the pubertal growth spurt in both boys and girls.[18] Further evidence supporting the role of estrogen in the male growth spurt came from the work of Caruso-Nicoletti and colleagues, who demonstrated that a 4-day infusion of estradiol 4 μg/d increased the ulnar growth velocity in 5 prepubertal boys.[18] The most convincing data supporting the importance of estrogen in the male pubertal growth spurt came from patients with familial male precocious puberty, in whom there is autonomous production of androgen from the testes. In these patients, therapy with an androgen antagonist alone is not sufficient to revert skeletal growth to a prepubertal rate. Once an aromatase inhibitor is added to the androgen antagonist to block conversion of androgen to estrogen, a prepubertal growth rate is once more achieved.[19] Since androgen antagonist therapy alone was not sufficient to slow the growth rate, this supported the notion that a major portion of the androgen-induced growth in boys was likely to be mediated via aromatization to estrogen.

ARE THERE ANY CONSEQUENCES TO LIFE WITHOUT ESTROGEN?

Up until 1994 it was impossible to conclude that estrogen played a major role in the growth of males since there were no human male models that lacked estrogen action. However, in 1994, a man with complete estrogen resistance, caused by a disruptive mutation in the estrogen receptor gene,[20] was described. For the first time we were provided with the unique ability to evaluate the role played by androgen alone, in the absence of estrogen action. The man with estrogen resistance experienced normal prepubertal growth and normal onset of secondary sexual characteristics. He achieved his midparental target height (5 ft 10 inches) at age 16 years. Despite full masculinization, however, epiphyseal fusion had not occurred and, consequently, he continued to grow. At 28 years, he was 6 ft 8 inches with a bone age of 15 years, and he was growing at a growth velocity of approximately 1 cm/y.[20] One year later, a man with the identical phenotype was described.[21] The striking feature of this 24 year old also was tall stature and continued linear growth as a result of delayed skeletal maturation. He was 6 ft 8 inches with a bone age of 14 years at chronologic age 24 years. This man had a disruptive mutation in the aromatase gene and an inability to convert androgen to estrogen. Within 6 months of therapy with congugated estrogens (0.3 mg/d increased to 0.75 mg/d over the first year), linear growth ceased and his epiphyseal growth plates fused.[22] A second male with aromatase deficiency, with the same skeletal phenotype, also has been described (Table).[23]

Table
Syndromes of Estrogen Deficiency in Males

	Estrogen Resistance **Case**[20]	Aromatase Deficiency **Case 1**[21]	**Case 2**[23]
Age (y)	28	24	38
Height (cm)	204	204	190
Upper/lower ratio	0.88	0.84	0.85
Tall stature	yes	yes	yes
Continued linear growth	yes	yes	yes
Eunuchoid habitus	yes	yes	yes
Bone age	delayed	delayed	delayed
Bone mineral density	reduced	reduced	reduced

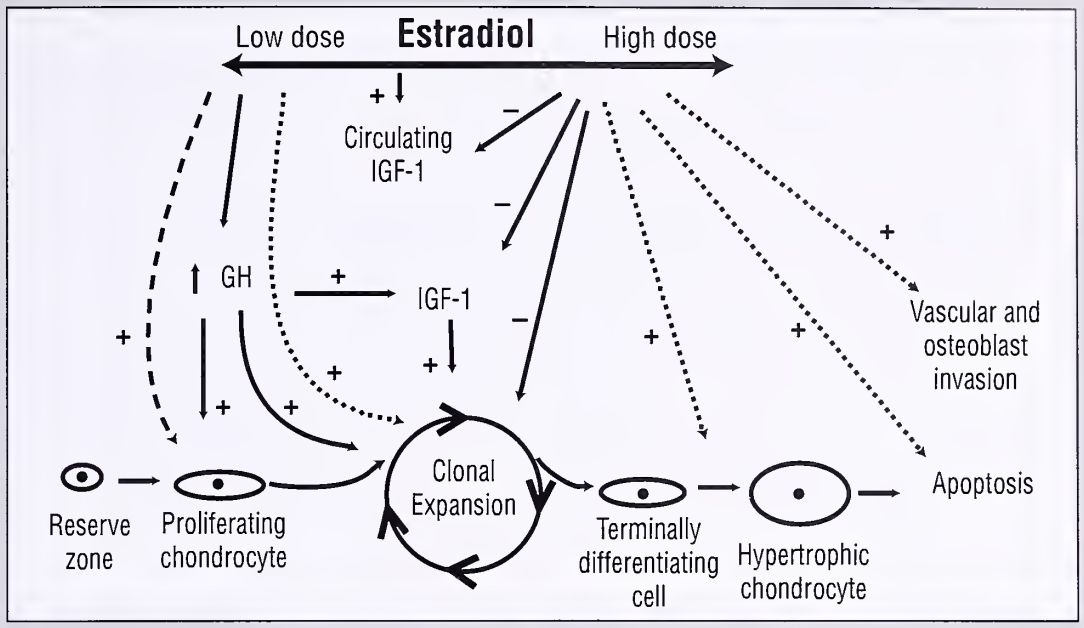
It is clear from the syndromes of estrogen deficiency that androgen, in the absence of estrogen, is relatively ineffectual in epiphyseal maturation.[24] However, it appears that androgen, in the environment of severe estrogen deficiency, is able to sustain linear growth despite arrested skeletal maturation. The estrogen-resistant and 2 aromatase-resistant males achieved their genetic potential for height at a normal age of 16 to 17 years, rather than at a later age, as would be expected with hypogonadal individuals. A possible explanation for the observed growth is that androgen, if not aromatized to estrogen, can stimulate growth directly at the level of the epiphyseal chondrocyte. In support of this are the observations made by Keenan and colleagues. They demonstrated that in short boys with delayed puberty, 5-dihydrotestosterone, a metabolite of testosterone and nonaromatizable androgen, induced and maintained an accelerated growth rate in spite of a 50% decline in integrated GH concentration and no change in IGF-1 level.[25]

All 3 males who lacked estrogen action have eunuchoid body proportions (Table), indicating relatively poor spinal growth (which is largely dependent on sex steroids) compared with limb growth. While it is tempting to speculate about the growth spurt of these individuals, there is insufficient longitudinal growth data on any of them to comment on the presence or absence of a growth spurt. Recently, a male infant with aromatase deficiency was described, and it will be highly informative to carefully follow his growth during his pubertal years.[26]

ESTROGEN ACTION AT THE GROWTH PLATE

As our clinical understanding increases, there is still much to learn about the mechanism of estrogen action at the growth plate. The growth plate is made up of chondrocytes, which are organized into layers. At the distal epiphyseal ends, the chondroblast progenitor cells occur singly or in small clusters to form

Figure 4



Proposed mechanism of action of estrogen at the level of the growth plate. The solid arrows represent data that have been demonstrated and the interrupted arrows represent other possible effects. Refer to text in article. GH, growth hormone; IGF-1, insulin-like growth factor 1.

the reserve zone. The next zone is the proliferative zone, in which the chondrocytes undergo clonal expansion and form discrete columns. The proliferative chondrocyte then undergoes terminal differentiation to form the hypertrophic chondrocyte. The hypertrophic chondrocytes secrete matrix, which undergoes mineralization. The hypertrophic chondrocytes then undergo apoptosis, and finally there is vascular and osteoblast invasion, which reduces the size of the growth plate.

Figure 4 represents some of our understanding of estrogen's role in growth. Low-dose estrogen increases GH secretion and stimulates growth. According to the dual effector theory of Green and associates,[27] GH primes the resting chondrocyte, preparing it for clonal expansion under the influence of IGF-1. However, GH receptors are not confined to the resting chondrocytes in the reserve zone, and GH and IGF-1 have been shown to exert their effect at each stage of differentiation.[28] The presence of estrogen receptor α in all populations of chondrocytes suggests that estrogen may have a direct role on the chondrocyte to stimulate growth.

High doses of estrogen inhibit growth by decreasing IGF-1 and inhibiting cell proliferation in the hypertrophic zone. The inhibition of clonal expansion by estrogen is not overcome by the addition of GH or IGF-1, suggesting that it is mediated directly by estrogen.[29] High-dose estrogen also may inhibit growth by inducing terminal differentiation of proliferating chondrocytes, apoptosis of hypertrophic chondrocytes, and vascular and osteoblast invasion into the growth plate.

CONCLUSION

Estrogen is only one of many important factors involved in chondrocyte growth and differentiation. Other critical factors include androgens, thyroid hormone, vitamin D, Indian hedgehog protein, and parathyroid hormone receptor protein. Despite our limited knowledge of the mechanisms at the level of the growth plate, we now appreciate the critical role that estrogen plays in the growth of both females and males.

REFERENCES

1. Zondec B. *Lancet* 1936;2:842-847.
2. Fancher TK. *Endocrinology* 1932;16:611-615.
3. Kirkland OL, et al. *Proc Staff Meet Mayo Clin* 1936;11:121-122.
4. Crawford JD. *Pediatrics* 1978;62:1189-1195.
5. Reyersbach GC. *AJDC* 1957;94:453-454.
6. Drop SL, et al. *Endocr Rev* 1998;19:540-558.
7. Yalow RS, et al. *Obes Res* 1996;4:583-600.
8. Glick SM, et al. *Nature* 1963;199:784-788.
9. Frantz AG, et al. *J Clin Endocrinol Metab* 1965;25:1470-1480.
10. Wiedemann E, et al. *J Clin Endocrinol Metab* 1972;34:51-58.
11. Wilkins L. *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence.* 3rd ed. Springfield, Ill: Charles C Thomas; 1965:25.
12. Ross JL, et al. *N Engl J Med* 1983;309:1104-1106.
13. Mauras N, et al. *J Clin Endocrinol Metab* 1989;69:1053-1058.
14. Copeland KC. *J Clin Endocrinol Metab* 1988;66:1278-1282.
15. Zachmann M, et al. *J Pediatr* 1986;108:694-697.
16. Prader A, et al. *Helv Paediatr Acta* 1977;37(suppl):1-44.
17. Conte FA, et al. *J Clin Endocrinol Metab* 1994;78:1287-1292.
18. Caruso-Nicoletti M, et al. *J Clin Endocrinol Metab* 1985;61:896-898.
19. Laue L, et al. *N Engl J Med* 1989;320:496-502.
20. Smith EP, et al. *N Engl J Med* 1994;331:1056-1061.
21. Morishima A, et al. *J Clin Endocrinol Metab* 1995;80:3689-3698.
22. Bilezikian JP, et al. *N Engl J Med* 1999;339:599-603.
23. Carani C, et al. *N Engl J Med* 1999;337:91-95.
24. Frank GR. *Acta Paediatr* 1995;84:627-630.
25. Keenan BS, et al. *J Clin Endocrinol Metab* 1993;76:996-1001.
26. Deladoey J, et al. *J Clin Endocrinol Metab* 1999;84:4050-4054.
27. Green H, et al. *Differentiation* 1985;29:195-198.
28. Hunziker EB, et al. *J Clin Invest* 1994;93:1078-1086.
29. Saggese G, et al. *Acta Paediatr Suppl* 1993;82(suppl 391):54-59.
30. de Waal WJ, et al. *J Clin Endocrinol Metab* 1996;81:1206-1216.

A Letter to Our Readers

March 15, 1985

Dear Colleagues:

The Editorial Board is pleased to introduce the inaugural issue of *GROWTH, Genetics & Hormones,* a publication for academicians and practicing physicians who are interested in these important areas of medical practice. We are pleased to welcome you as a reader and invite you to participate as a reader and as a correspondent.

Normal and abnormal growth, genetically determined conditions, and the overall development of children are important aspects of pediatric practice. Hormonal production is essential in growth and development. It is probable that these areas will assume even greater prominence because pediatricians are showing greater interest in growth and development as immunizations and antibiotics diminish the incidence of infectious disease, as greater numbers of children with leukemia and other cancers enter sustained remissions, and as growing numbers of handicapped infants with congenital anomalies survive.

It is also well recognized that the literature concerning these topics is voluminous. Therefore, *GROWTH, Genetics & Hormones* was developed, primarily to provide a close look at current—and often controversial—topics in endocrinology, genetics, and metabolism and their potential clinical applications. To ensure that this goal is met now and in the future, several nationally and internationally respected authorities in genetics, endocrinology, anthropometrics, pediatrics, pharmacology, and metabolism have agreed to serve on the Editorial Board.

The eminent investigators who have agreed to serve as Associate Editors are: Dr. Jürgen Bierich of the University of Tübingen, West Germany; Dr. Judith Hall of the University of British Columbia Medical School; Dr. Fima Lifshitz of Cornell University School of Medicine; Dr. David Rimoin of the University of California, Los Angeles; Dr. Alan Rogol of the University of Virginia School of Medicine; and myself. You will meet each of the Board members in the early issues of *GROWTH, Genetics & Hormones.*

The editorial content of this quarterly publication was chosen with your interests in mind. This issue, for example, features an article about the incidence of growth hormone deficiency, a review of growth hormone physiology and pathophysiology, and a summary of a recent conference concerning the psychosocial aspects of growth delay. These are scientific, timely, and representative of the topics that *GROWTH, Genetics & Hormones* will address.

Abstracts of pertinent articles and reports will appear in each issue. In the future, the abstracts will serve as "mini-reviews" and integrate multiple reports on a particular topic.

We are pleased to present this inaugural issue to you. We welcome your readership and look forward to hearing from you about *GROWTH, Genetics & Hormones.* We would also appreciate your filling out the enclosed reply card to let us know of your initial interest.

On behalf of the Editorial Board: Sincerely, Robert M. Blizzard, MD, Professor and Chairman, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville.

A Second Letter to Our Readers

March 15, 2000

Dear Colleagues:

Fifteen years have passed since the previous letter was written. Hopefully, *GROWTH, Genetics & Hormones* has served you in accord with the substance of that letter. The Editorial Board is proud of this publication and its accomplishments.

Several members of the initial Editorial Board remain—specifically, Dr. Judith Hall, Dr. Fima Lifshitz, and myself. The other current members are Dr. William Horton, Director of Research, Portland Shriners Hospital; Dr. William Clarke, Professor of Pediatrics, University of Virginia School of Medicine, and Dr. Allen Root, Professor of Pediatrics, University of South Florida. They have served for at least the last 6 years. Other members who served for an extended period in the interim between the writing of these 2 letters are Dr. Jürgen Bierich of Tübingen; Dr. James Tanner of London; Dr. Jean Claude Job of Paris; and Dr. Alan Rogol of Charlottesville.

In the 15 years since the initial publication, there have been more than 100 lead articles, 20 reviews of important international or national meetings, and more than 700 abstracts of pertinent articles from the literature with editorial comments. The Editorial Board expresses its gratitude to Genentech, Inc. for sponsoring this continuing education publication under an unrestricted educational grant awarded to the University of Virginia. Distribution is to endocrinologists in pediatrics and internal medicine, geneticists, nurses, and other physicians who have requested issues. Distribution to pediatric endocrinologists in Europe was discontinued for budgetary reasons after 13 years. We are pleased to announce that beginning with this issue, members of the European Pediatric Endocrine Society are again receiving their copy, which is now sponsored by Schwarz Pharma AG. Thanks to Schwarz Pharma AG, it is probable that the 3 issues that will be published in 2000 will increase to 4 issues in 2001.

Letters to the Editor are encouraged, as stated in the letter of March 15, 1985. Letters can be sent to the Editor-in-Chief at 1224 West Main Street, Suite 701, Charlottesville, VA 22903.

The Editorial Board wishes to hear from you concerning all aspects of *GGH.* If your issue does not arrive as expected, please write to the Editor-in-Chief at SynerMed, 405 Trimmer Road, Califon, NJ 07830. Please let the Editorial Board hear from you so we can better serve you.

On behalf of the Editorial Board: Sincerely, Robert M. Blizzard, MD, Professor and Chairman Emeritus, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville.

Ethical Issues in Growth Hormone Therapy: Where Are We Now?

David B. Allen, MD
Norman C. Fost, MD, MPH
University of Wisconsin
Madison, Wisconsin

BACKGROUND

In 1991, a group of pediatric endocrinologists, ethicists, economists, and psychologists convened to address ethical questions arising from expanding use of recombinant human growth hormone (GH).[1] These included: (1) If GH was proven effective at improving the height of children without GH deficiency (GHD), is the diagnosis of GHD morally relevant in determining entitlement to GH treatment? (2) To what extent should the treatment of short stature (SS) be considered a medical problem requiring or justifying medical treatment? Much of the debate at that time centered around the argument put forth by Drs. Allen and Fost in a prior publication that GH responsiveness rather than GHD should guide access to GH.[2] Others at that conference countered that patients with GHD had a *disease* deserving *treatment*, while children with SS from other causes did not have a disease (at least not that of GHD) and to treat them is *enhancement* and not therapy.

The intervening years have not resolved this debate and new, equally important and problematic issues have arisen. The addition of Turner syndrome (TS) and chronic renal insufficiency (CRI) with SS to the list of approved indications for GH treatment validated the notion that GH effectiveness and not the underlying etiology of SS is the relevant variable in determining the appropriateness of treatment. With GH treatment no longer constrained by the treatment/enhancement distinction, numerous other SS conditions appeared as candidates. Further, new knowledge about important non–growth actions of GH and the importance of GH treatment of severe GHD in adulthood spawned interest in possible uses of GH in other types of SS in childhood. As GH utilization continues to expand, concerns and questions have been renewed about excessive expenditures of limited health-care resources. An additional question is, Can increased height measure the value of GH treatment and, if not, what can? To address these issues, 25 endocrinologists, ethicists, psychologists, and insurance representatives convened in October 1999 for a second ethics conference in Madison, Wisconsin. Adhering to the maxim that "good ethics begins with good facts," an initial session focused on current knowledge regarding the benefits and burdens of GH treatment in children with GHD, TS, and Prader-Willi syndrome (PWS), and in adults with GHD. A second session analyzed how decisions guiding access to GH treatment are and should be made from the perspectives of local insurance providers, planners of the Oregon Health Plan, and individuals considering the national health-care economy. The group then debated the proper endpoint of GH treatment in children: Should it be the maximal attainable height or simply "normal" height, or should psychosocial adaptation, rather than height, be the appropriate endpoint? Finally, conceptual issues raised by the initial conference[1] were revisited: Specifically, is the treatment/enhancement distinction helpful in guiding access to GH treatment, and how should the use of GH treatment be determined in the context of the American health-care system?

SCIENTIFIC ISSUES

Dr. Margaret MacGillivray, a pediatric endocrinologist, presented current data describing adult heights of hypopituitary children treated recently with GH (– 1.5 to – 0.7 SDS). The result is a marked improvement in final height compared with former treatment with pituitary-derived GH (– 4.7 to – 2.0 SDS). She predicted further improvements with earlier treatment and improved pubertal GH replacement.

Dr. David Sandberg, a psychologist, then addressed perhaps the most complex and contentious topic of the conference: What do we know about the quality-of-life (QOL) benefits of GH-increased final height? While acknowledging the daunting methodologic challenges of such research, he reported that his and other studies failed to show a relationship between adult height and QOL, suggesting that maximizing adult height outcomes does not automatically translate into improved QOL outcomes. Such observations, if confirmed, will have clear relevance to questions regarding both termination of GH therapy and GH treatment of non-GHD children for presumed psychosocial benefit.

The successful treatment of TS girls with GH is now regarded as standard practice, but is the benefit of treating these children truly clinically significant? Dr. Ron Rosenfeld, a pediatric endocrinologist, was assigned the "pro" position for this debate. He presented evidence that GH accelerates growth and improves final height in TS, that this outcome can be achieved without excessive delay in pubertal development, and that GH is safe for these patients. Anecdotal experience suggested a beneficial effect of GH therapy on QOL in children and young adults with TS, but studies assessing impact of growth-promoting therapy on psychosocial function are lacking. Conclusive data will be extremely difficult, if not impossible, to obtain in the current environment. Dr. Harvey Guyda, also a pediatric endocrinologist, was assigned the "con" position. He argued that the desired outcome for most patients (achie-

ing a "normal" height) does not occur for the majority of TS girls treated with GH. The median final height achieved by patients in the Canadian randomized controlled study is only – 2.3 SDS. While some individuals have shown dramatic responses, only ~50% of those in the Canadian study can expect a height benefit >5 cm. Further, Guyda emphasized that treatment has not been proven to lead to improved psychosocial functioning. A challenge remains to determine methods to identify the TS patients who are most likely to benefit from prolonged GH treatment.

The determination of a responsible endpoint for GH therapy for growth promotion in all conditions remains problematic. Dr. David Allen, a pediatric endocrinologist, proposed that treatment should be stopped when the height is within the normal adult range. This represents not only a successful therapeutic outcome but also a more reasonable allocation of resources and preservation of a proper goal for the medical profession in the treatment of SS. On the other hand, Allen stated, since many children with unexplained isolated GHD display normal GH secretion after puberty, continuous treatment to maximal height may include years of unnecessary treatment, during which time GH therapy becomes increasingly expensive and, simultaneously, less effective as final height is approached. From an ethical standpoint, Allen stated, promoting additional growth within the normal adult range could be viewed as enhancement and not treatment. Dr. Michael Kappy, a pediatric endocrinologist, countered that using the lower range of adult height as a goal represented a gender-biased definition of handicap, since demands for daily living (eg, reaching for objects on a high shelf) are not gender-specific functions. Instead, the criterion for discontinuing GH should be purely physiologic, ie, how tall the child would have grown if he or she did not have GHD. He argued that this approach was less arbitrary and reduced the risk that the physician would need to make value judgments.

Novel uses of GH treatment add complexity in identifying appropriate recipients and in determining appropriate outcomes for judging the value of such treatment. For example, adults with severe GHD have abnormal body composition, deficient bone mineral density (BMD), lipid abnormalities, and a decrease in QOL. Dr. David Cook, an internist and endocrinologist, emphasized that while several such entities appear amenable to GH replacement therapy, the focus of insurance companies is favorable changes in mortality figures and reduced bone fracture rates, rather than QOL or metabolic issues. However, awareness of these non–growth or metabolic effects of GH has raised interest in the effect of GH therapy on body composition and physical function in disabled children, such as those with PWS. Dr. Aaron Carrel, a pediatric endocrinologist, reviewed data showing that in PWS children GH therapy improves body composition such as reducing body fat and increasing lean body mass, BMD, fat oxidation, and energy expenditure. Most importantly, physical strength and agility are improved. These benefi-

cial effects were viewed by families to be as important or even more important to the well-being of the child than gain in height. The view of many insurance companies may not be in accord with the views of physicians who are treating these children.

ETHICAL, SOCIAL, ECONOMIC, AND POLICY ISSUES

Appropriately, the conference then turned its attention to ethical, social, economic, and policy issues regarding access to GH therapy. The medical directors of 3 Wisconsin-based HMOs presented their organizations' policies on paying for GH treatment and explanations of how such decisions are made. While all 3 HMOs seemed to use similar approaches, their conclusions were disturbingly divergent. One provided full reimbursement for treatment for GHD and TS; one required 50% copayment; and the third paid nothing for either indication. They all claimed to rely on medical necessity as the central criterion for resolving such questions, but the definition of this term was unclear.

Mark Pauly presented an economist's perspective, one based on the assumptions that GH was safe, effective, and available in unlimited supply, and that market conditions were ideal, including that potential purchasers had full knowledge of the facts. In such a system, the most generous insurance packages would probably cover treatment for very short children with GHD, but that this would be less likely for children already in the normal range or for conditions for which treatment produced only minimal increases in height. He thought it likely that there would be public support to subsidize only the most severely affected children.

With regard to accepting private purchase of GH for children who were not severely affected, this appeared compatible with existing notions in the United States of tolerating individual discretion in spending earned income for health matters, particularly if these decisions did not cause severe harm to those who could not afford treatment. Given the apparently modest gains produced by GH treatment in most non-GHD children, Pauly thought it unlikely that there would be severe overall harm. To him the inequality likely to be produced by such private purchases seemed trivial compared with other consequences of inequality of wealth that are currently prevalent.

Philosopher Paul Menzel described the approach of the Oregon Health Plan with regard to access to GH by its Medicaid population. Oregon has identified 743 "treatment/condition pairs," and currently prioritizes funding down to No. 574. Pituitary dwarfism is included (No. 499), as is GH therapy for TS (No. 506). GH treatment is not supported for any other conditions that presumably fall into the generic category, listed at No. 736, which is titled, "Endocrine or metabolic conditions with no effective treatment or where no treatment is necessary." (However,

which criterion was considered relevant in the exclusion is unclear.) Menzel suggested that from the perspective of social justice in access to health care, the apparently marginal benefit of GH treatment of most non-GHD children with regard to the modest increase in height should not cause great alarm. To the contrary, the more important question might be whether the benefits that accrue to patients with TS can be justified in the Oregon plan when one considers other possible use of the funds.

Pediatrician Douglas Diekema questioned the claim that treatment with GH is a net benefit, particularly for children without GHD. He focused on the ambiguity of psychosocial benefit and thought more consideration should be given to the potential psychological harm of treatment, which is supported by some studies. This could occur from an implied message to children that their parents are unaccepting of them. This may be particularly problematic when treatment produces little or no increase in height. He also pointed out that treatment of these children does not make them tall but only less short.

Dr. Norman Fost, a pediatrician and ethicist, reviewed the hazards of trying to resolve questions of access by relying on traditional distinctions made between health and disease and between treatment and enhancement. He argued that some conditions are clearly diseases, such as the persistent vegetative state, and yet might not warrant expensive prolonged treatment. Similarly, some conditions are clearly not diseases, such as pregnancy, and yet attract wide support for treatment to be included in a basic benefits package. He applied similar analyses to treatment versus enhancement distinctions, stating that some clear treatments did not warrant funding and some clear enhancements did. The latter could include bringing "normal" short children into the normal height range.

Philosopher Allen Buchanan discussed GH as a paradigm of "expansive biotechnologies," which refers to technologies that begin as clear medical treatments and then are found to offer benefits that do not clearly belong in the health-care system. Few would dispute that pituitary dwarfism is a medical problem warranting medical treatment, but many of the newer applications of GH, such as producing marginal increments in height or improving strength or slowing the aging process, are not so clearly defined as medical problems. He compared GH with other technologies and drugs, including artificial insemination and Prozac. These began as treatments for medical problems but expanded to much larger markets that involved problems that were less clearly medical. He expressed concern that these expanding uses for very expensive technologies "might erode our society's already shaky commitment to the right of an adequate level of health care for all."

Philosopher Dan Brock concluded the conference with several summary observations. He cited the need for a clear formulation of the ultimate endpoints we seek from GH treatment. He also cited the lack of data on the degree to which current endpoints are achieved. He stated that height itself cannot be an appropriate endpoint because "it is only instrumental to improvements in patients' quality of life." He urged that future studies focus on QOL gains since coverage by insurance would be difficult to justify without clearly established QOL benefits. He concurred with Dr. Fost that whether SS is a disease, and whether use of GH is characterized as treatment or enhancement, would not resolve the critical questions.

In reviewing Dr. Pauly's presentation, he noted that health-care markets do not function well, and the inequities in income distribution may be difficult to justify, so that leaving access to the market would be difficult to support as a matter of social justice. On the other hand, Dr. Pauly acknowledged the practical and theoretical difficulties in limiting the freedom of those with discretionary money to spend it as they wish. Brock reminded the group of the axiom that one cannot go from an "is" to an "ought," so that the present arrangements in income distribution and health care cannot be presumed to be fair. He acknowledged that we do not yet have an adequate framework for resolving problems of access and rationing.

CONCLUSION

A decade ago, Allen and Fost asked whether GH therapy would become a panacea or Pandora's box. The question remains unanswered. Viewpoints expressed at this conference suggest that while some issues have been clarified, new questions have arisen. While GH can increase final adult height in some patients, the effect on their QOL remains unclear. While the safety of long-term GH therapy is clearer, the costs continue to be high, and new indications, such as treatment for metabolic reasons, and new questions about who should have access to GH and who should pay remain unanswered. The other questions asked by Drs. Allen and Fost in 1991 also remain unanswered. Continued frequent discussion of these issues by physicians, ethicists, health economists, and others together is essential if responsible and equitable use of GH during the next decade is to occur.

REFERENCES

1. Allen DB, Fost NC. Access to treatment with human growth hormone: proceedings of a conference. *GROWTH, Genetics, & Hormones* 1992; 8 (supplement).

2. Allen DB, Fost NC. Growth hormone therapy for short stature: panacea or Pandora's box? *J Pediatr* 1990;117:16-21.

PUBLICATION NOTE

The complete proceedings of the conference, including presented papers and an edited transcript of the discussions, will be published as a supplement to *Pediatrics*.

Effects of Recombinant Leptin Therapy in a Child With Congenital Leptin Deficiency

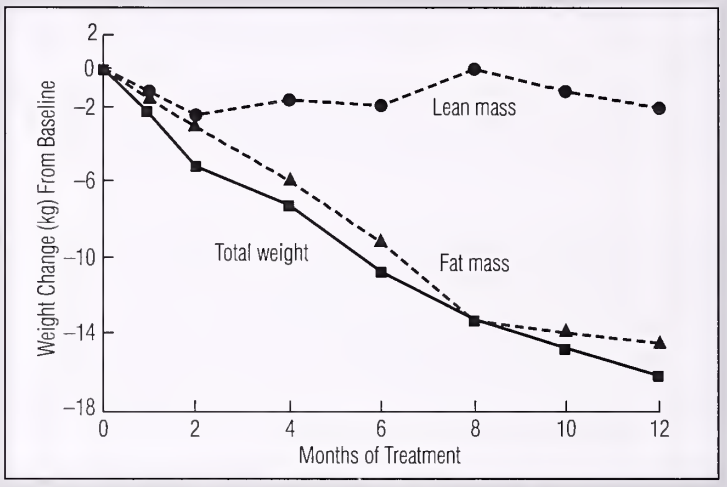
A patient is reported with early onset morbid obesity due to leptin deficiency. The effects of trial therapy with recombinant human leptin also are reported. This patient was normal weight at birth but began growing excessively at 4 months of age (Figure). She had marked hyperphagia and was constantly hungry, demanded food, and was very disruptive when food was denied. She was born in a highly consanguineous family of Pakistani origin. Her nonobese parents were first cousins. She gained excess weight throughout her life, exceeding the 100th percentile and reaching 94.4 kg at age 9 years. Her height was 140 cm (91st percentile). At that time, she was treated with recombinant human leptin, administered subcutaneously daily at 0.028 mg/kg of lean mass for 12 months. The patient lost weight immediately with treatment and her weight decreased 16.4 kg at a rate of 1 to 2 kg/mo. Her height remained at the same percentile throughout the treatment. The body composition measurements revealed a decrease in the body fat by 15.6 kg (95% of the total weight loss). Simultaneously, leptin treatment was associated with a decreased consumption of food and a marked change in eating behavior. The injections were tolerated well.

Farooqi IS, et al. *N Engl J Med* 1999;341:879-884.

Editor's comment: *This patient with congenital leptin deficiency and morbid obesity is the first one treated with recombinant human leptin. The results were impressive, confirming the importance of this hormone in the regulation of body weight and appetite. This patient and her response to treatment, which replaced the hormone that was lacking, demonstrated major differences between the human condition from those found in mice with* OB *and* DB *mutations leading to leptin deficiencies. The reader is encouraged to review the article as well as the accompanying editorial in this issue by Drs. Michael Rosenbaum and Rudolph Leibel* (N Engl J Med 1999;341:913-914). *I also recommend the review published in* GGH *(Vol. 14, No. 3, October 1998) of the leptin abnormalities reported to occur in humans that are associated with this type of obesity in children.*

Fima Lifshitz, MD

Figure



Reprinted with permission from Farooqi IS, et al. *N Engl J Med* 1999;341:879-884.

Recombinant Leptin for Weight Loss in Obese and Lean Adults: A Randomized, Controlled, Dose-Escalation Trial

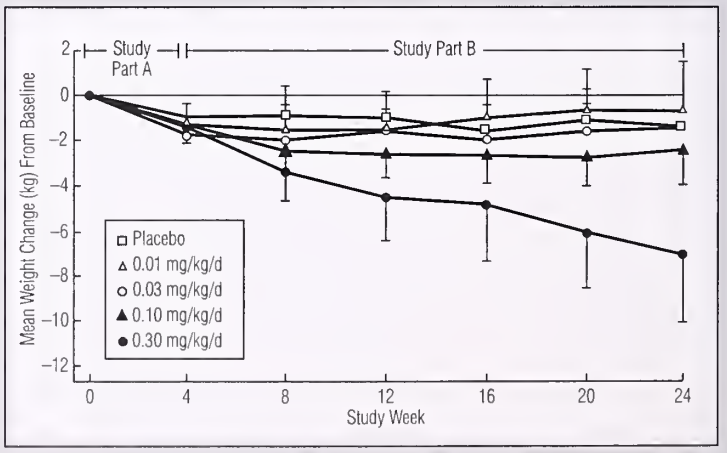
The protein hormone leptin is produced by adipocytes and regulates the amount of body fat. In animals, leptin also has been found to regulate energy intake. This study reports the effects of different daily doses of leptin in lean and obese individuals. Fifty lean and 73 obese subjects self-injected either 0.01, 0.03, 0.10, or 0.30 mg/kg/d of recombinant human leptin subcutaneously daily for 4 weeks. The obese subjects were given leptin for an additional 20 weeks. All also were placed on an exercise routine and reduced calorie diet. After the first 4 weeks of treatment, both the lean and obese subjects lost weight (–0.4 to –1.9 kg). After 20 additional weeks, the obese group lost additional weight from –1.9 kg, for the 0.10-mg/kg dose, to –7.1 kg for the 0.30-mg/kg dose (Figure). The weight loss was mainly body fat.

Heymsfield SB, et al. *JAMA* 1999;282:1568-1575.

Editor's comment: *This is the first dose-response study showing that increasing body weight loss is associated with increasing doses of leptin. Furthermore, the composition of weight loss was mainly body fat; thus, lean body mass was preserved. These results suggest that leptin may be appropriate for treating obesity in certain individuals. However, the results may not*

Figure

Pattern of Weight Change From Baseline to Week 24 in Obese Subjects Who Received rLeptin*



*rLeptin refers to recombinant methionyl human leptin.
Error bars indicate SEM; gray line indicates baseline.

Reprinted with permission from Heymsfield SB, et al. *JAMA* 1999;282:1568-1575.

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
SCHOOL OF MEDICINE

GROWTH, Genetics, & Hormones
Volume 16, Number 1

Post-Program Self-Assessment/CME Verification Answer Sheet

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Answer each of the questions or statements contained in the Post-Program Self-Assessment/Course Evaluation. There may be more than one correct answer. Participants are encouraged to refer to the related articles for evaluation of their responses. To receive Category 1 credit, complete the self-assessment exam/course evaluation and record your responses on the answer sheet. Enclose a check for $20 made payable to the University of Virginia or complete credit card information. Mail the answer sheet and fee to:

GGH
PO Box 40
Garwood, NJ 07027-0040

A certificate will be mailed to you. This enduring material was approved for credit March 2000. Eligibility for credit expires March 2003.

Name (print clearly) Degree Specialty

Street

City State Zip

Phone SS No.

Amount enclosed: _____ ❑ Check

❑ Credit Card (circle one) VISA MC

Credit Card Number: _____ Exp Date: _____

Signature (credit cards only): _____

Record your answers by circling the appropriate letter(s) for each question.

1. a b c
2. a b c
3. a b
4. a b c d
5. a b c d

Course Evaluation

5 = Excellent 4 = Above average 3 = Good 2 = Below average 1 = Poor

Please evaluate this course with respect to the following:

1. Educational value of newsletter	5	4	3	2	1
2. Clinical relevance of articles	5	4	3	2	1
3. Newsletter style/format	5	4	3	2	1
4. Length of articles	5	4	3	2	1
5. Were the educational objectives met?	5	4	3	2	1

6. In your opinion, how could this newsletter be improved?

_____

_____

_____

be totally attributed to leptin because all subjects were placed on exercise and a reduced calorie diet while being treated with leptin. The investigators should have had a similar treatment group without the corresponding exercise and dietary prescription to clarify the therapeutic value of this hormone in treating obesity. This would have factored out the effects of corresponding treatments on body weight loss. However, from the results reported leptin should not be considered a panacea for the treatment of obesity. High doses of the hormone were necessary to reduce weight, denoting leptin resistance. I can foresee being forced into treating obesity with leptin as we were for treating short stature with GH therapy.

Fima Lifshitz, MD

Evidence Supporting an Adipo-Leptin-Growth Hormone Axis in Obesity-Related Hyposomatotropism

Roemmich et al review the evidence that the reduced GH secretion observed in obesity may be related to leptin physiology. The hypothesis is presented in the figure and its legend, which are reproduced here.

In advancing this hypothesis, the authors review the neuroendocrine control of GH secretion; alterations in GH release during childhood, adolescence, and adulthood; the influence of GH secretion on body composition; the altered neuroendocrine control of GH secretion in obesity; leptin physiology; the evidence of an inhibitory role for leptin on GH secretion; and the influence of GH on leptin secretion.

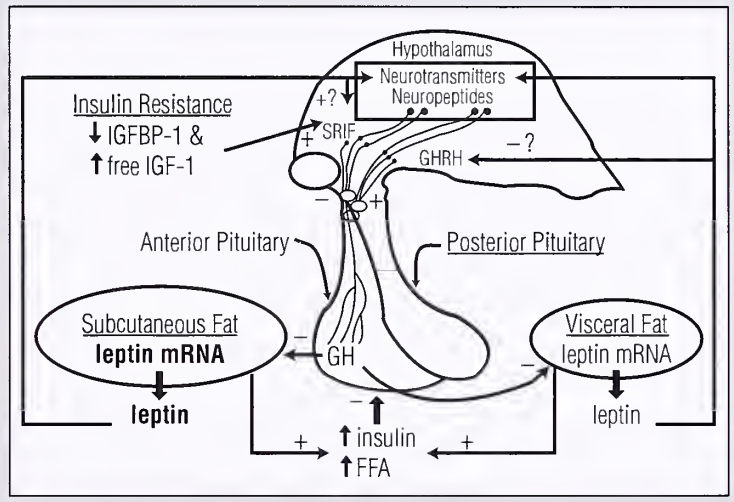
The authors conclude that GH secretion is impaired in obese adults and children but the physiologic mechanisms producing hyposomatotropism remain unclear. Apparently, metabolic signals relay information regarding the body composition and the fat distribution to the hypothalamus and pituitary. Leptin is a logical choice as a messenger of the fat stores because it is secreted directly by the adipocytes and leptin receptors are located in the pituitary and the hypothalamus, including GH-releasing hormone neurons. However, the evidence is not yet convincing enough to conclude that leptin plays a primary role in the modulation of the neuroendocrine GH axis in obesity.

Roemmich JN, et al. *The Endocrinologist* 1999;9:424-430.

Editor's comment: *This paper is a good, timely review of the physiology of and interaction among GH, leptin, insulin, and GH-releasing hormone. The article is brought to the attention of the readers of* GGH *as the hypothesis presented is well worth considering. It may be totally true, partially true, or not at all true. Further studies and reflection on these studies are needed.* GGH *wishes to expose you to concepts as well as facts. Your attention is called to an article published recently in* GGH *entitled "Molecular Physiology of Leptin and Its Receptor" (Zhang Y, Leibel RL.* GGH *1998;14:2) that was an excellent review of the facts known as of the date of publication.*

Fima Lifshitz, MD

Figure



Schematic of the hypothesized adipo-leptin-GH axis. Leptin is predominantly secreted from the subcutaneous fat depot. An accumulation of subcutaneous fat increases serum leptin concentrations that feed back to the hypothalamus and perhaps the pituitary. Leptin receptors have been located in the human hypothalamus and in the rat, but not the human pituitary. Acting through as yet unknown neurotransmitter and neuropeptide mechanisms, leptin could increase somatostatin (SRIF) tone or inhibit GH-releasing hormone (GHRH) tone, resulting in a reduced somatotropic response at the pituitary. Neuropeptide Y modulates the leptin-induced reduction in GH secretion in fasted rats, but there is no evidence that neuropeptide Y modulates the reduced GH secretion caused by obesity. Leptin also may directly inhibit GHRH secretion because leptin receptors are expressed in GHRH neurons of the rat. However, leptin is not necessary for reducing GH secretion. Both the *ob/ob* mouse and humans with mutated leptin genes are obese, and GH release is reduced in the absence of leptin.

The metabolic hypothesis for obesity-induced reductions in GH secretion is better established. An accumulation of fat in both the subcutaneous and visceral fat depots is associated with an increase in serum insulin and free fatty acid (FFA) concentrations, which act directly at the pituitary to inhibit GH secretion. Obesity (likely through its association with insulin resistance) also reduces IGF-binding protein 1 (IGFBP-1) concentrations, resulting in increased free IGF-1 concentrations, which may feed back to increase somatostatin tone. Regardless of the mechanism, the reduction in GH secretion results in a reduction in GH-mediated lipolysis, further gains in subcutaneous and visceral fat, and further increases in serum leptin, insulin, lipid, and free IGF-1 concentrations.

Blood, Sweat and Tears—or Is It Teeth, Sweat Glands, and Hair?

A very interesting story has unfolded over the past few years regarding the pathogenesis of hypohidrotic ectodermal dysplasia (HED). The gene harboring mutations responsible for the X-linked form of this disorder, which is characterized by abnormal formation of teeth, hair, and eccrine sweat glands, was identified by positional cloning about 3 years ago. Designated *ED1*, it encodes a transmembrane protein, called ectodysplasin (Eda), that contains a collagen-like region in its extracellular domain. This region is thought to mediate formation of trimers as the extracellular portions of the molecules extend from the cell surface.

Mutations in the mouse homologue of human *ED1* have been found in a mouse mutant called tabby *(ta)*, which has a murine phenotype equivalent to HED. Since Eda appears to be involved in the induction of ectodermal placodes, which give rise to structures that fail to form in both HED and *ta,* Eda was proposed to function as a membrane-bound ligand, although the mechanism of signal transduction was not known. This speculation led to a search for an Eda receptor. The receptor was predicted to be encoded by an autosomal gene based on the existence of autosomal forms of HED that are clinically indistinguishable from the X-linked form. The search has now ended with the identification of a receptor for Eda.

The latest chapter of the story begins with a mouse mutant named downless *(dl)*. The close similarity between the *dl* and *ta* phenotypes suggested the possibility that *dl* encoded the Eda receptor. Positional cloning of *dl* by Headon and Overbeek revealed it to be a novel member of the tumor necrosis factor (TNF) receptor. This argues for its being the Eda receptor since TNF receptors typically bind trimeric ligands, the form proposed for Eda. Moreover, its expression pattern corresponds well to sites in developing skin, where ectodermal placodes form.

Armed with the mouse *dl* cDNA as a probe, Monreal et al quickly cloned the human *DL* gene. They next searched for *DL* mutations in HED patients with suspected autosomal inheritance. They identified *DL* mutations in 3 families with autosomal recessive HED and in 2 families with autosomal dominant HED. Two of the recessive families displayed consanguinity. As expected, the affected members were homozygous for the putative mutations and their parents were heterozygous for the mutations. Affected members in the third recessive family were compound heterozygotes. Mutations in the recessive families probably act through haploinsufficiency.

The mutation in one of the dominant families predicts a truncated protein lacking a key functional domain, the so-called death domain, which lies at the carboxyl terminal of the molecule. Trimerization of the cytoplasmic death domain is required for signal transduction, typically to transmit signals that bring about cell death. Such mutant proteins could participate in interactions of trimeric ligands with trimeric receptor molecules, but they would not transmit signals downstream. Thus, the mutation would act in a dominant negative manner, which would explain why this type of mutation is inherited as a dominant, while the other loss of function mutations are inherited as recessives.

Monreal et al noted that some families with HED do not map to either the *ED1* or the *DL* locus, implying the existence of at least a third HED locus. A mouse mutant named crinkled *(cr)* displays a phenotype very similar to that of tabby and downless. The crinkled gene has not yet been cloned, but it represents a good candidate for the third HED locus.

Another interesting aspect of this story is that although the Eda ligand is membrane bound, it contains an extracellular cleavage site for the secreted metalloprotease, furin. Thus, it is possible that the trimerized ectodomain of Eda is cleaved to produce a ligand that diffuses at least locally in search of its receptor. Such cleavage is characteristic of TNF ligands.

Headon DJ, Overbeek PA. *Nat Genet* 1999;22:370-374.
Monreal AW, et al. *Nat Genet* 1999;22:366-369.
Barsh G. *Nat Genet* 1999;22:315-316.

Editor's comment: *The evidence to date strongly supports the view that* DL *in humans (and* dl *in mice) is a receptor for Eda and that signal transduction involves trimerization of Eda and its receptor as occurs with other TNF:TNF receptor interactions. Membrane-bound Eda may be cleaved from the cell of origin to diffuse to the cells it influences. The evidence further suggests that the downstream signals are required for induction of ectodermal placodes, which give rise to teeth, hair follicles, and sweat glands.*

The unfolding of this story provides another good example of how human and mouse genetics are interrelated and how analysis of mutant phenotypes yields insight into normal biology. Also, given the fact that, even in adults, cells in hair follicles recapitulate the developmental program that produces hair in early life, it seems quite possible that this work will lead to advances in the treatment of hair loss and in hair removal.

William A. Horton, MD

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc. The information reflects the views of the editors and/or contributors and not necessarily those of the sponsor, grantor, or the publisher.

Published by:
Gardiner-Caldwell
SynerMed

405 Trimmer Road
PO Box 458
Califon, NJ 07830

The Rett Syndrome Gene Silences Many Other Genes

Rett syndrome is a common cause of mental retardation in females, with an incidence of 1 per 10,000. It is characterized by normal development until 6 to 18 months of age when there is regression, including loss of speech, hand-wringing, seizures, autism, ataxia, hyperventilation, and, often, growth retardation. The phenotype is very characteristic; however, most cases are sporadic and it is never observed in males. The hypothesis was that it was an X-linked dominant disorder, lethal in hemizygous males. Familial cases had been localized and linked to the tip of the long arm of the X chromosome in the Xq28 region. Amir et al have reported finding mutations in the *MECP2* gene in 6 sporadic cases and 1 familial case of Rett syndrome. The responsible gene, *MECP2*, encodes a methyl-CpG-binding protein that selectively binds CpG dinucleotides and mediates transcription from a variety of genes by repressing the interaction with histone deacetylases. Thus, the Rett syndrome gene is probably a key player in silencing other genes. In other words, the gene normally plays a role in assembling transcription silencing complexes. However, if these complexes are not working at a specific stage in development, then the genes will continue to make proteins that apparently clog up normal processes and lead to the intellectual degeneration of affected females. The recognition of the *MECP2* gene as being responsible for Rett syndrome is the first time a human disease has been determined to be caused by a defect in a protein that involves DNA methylation and, thus, when the protein is absent or not working, leads to abnormal chromatin packaging and abnormal gene expression. Interestingly, the gene is particularly expressed in the brain, and thus it would appear that the brain is particularly sensitive to an excess of transcribed proteins. Undoubtedly, there will be other such genes but it is a real breakthrough in understanding abnormalities in developmental time-specific processes.

Willard HF, et al. *Nat Genet* 1999;23:127-128.
Amir RE, et al. *Nat Genet* 1999;23:185-188.

Editor's comment: *The Human Genome Project is revealing many genes with no previously described homologies, as well as demonstrating many new, previously unknown processes. It is reassuring to have the gene responsible for a common syndrome defined, but surprising to find it affects many other genes in a specific developmental way. In the process of a child's development, there must be many other episodes of switching on and off. Interestingly, the mouse model for* MECP2 *deficiency also is X-linked and affected males do not develop at all since it leads to male lethality. Because the human cases are mostly sporadic, the effect of male lethality has not been observed. It seems quite possible that if affected girls could be recognized in the newborn period, some type of therapy could be developed.*

Judith G. Hall, OC, MD

2nd Editor's comment: *These articles describe a novel pathogenetic mechanism for genetic disease in humans. If the speculation proves correct, Rett syndrome results from at least partial failure of a global process that keeps transcription in check. As Amir, Willard, and their colleagues emphasize, much more work will be needed to prove the theory, and it will likely turn out to be much more complicated than outlined here. Nevertheless, it is an exciting development in medical genetics.*

William A. Horton, MD

Chromosome 7p Maternal Duplication With Features of Silver-Russell Syndrome

Maternally uniparental disomy of chromosome 7 is present in about 10% of Silver-Russell syndrome (SRS) individuals, suggesting there is a gene or genes that are imprinted on chromosome 7. Growth-related genes on chromosome 7, including *GRB10* (a growth factor receptor-bound protein), *EGFR* (epidermal growth factor receptor), and *IGFBP1* (insulin-like growth factor-binding protein 1), have all been suggested as candidate genes. However, molecular analysis of a duplication present in both mother and daughter who have SRS shows that it includes *GRB10* and *IGFBP1* (but not *EGFR*), suggesting that one or both of these are the culprits involved in the phenotypic effects.

The authors summarize:

> We have characterized a duplication of 7p12.1-p13 in a mother and daughter who both show features associated with SRS. It seems likely that a gene or genes contained within this region are responsible for at least some of the SRS features and that, in our patients, duplication of additional contiguous genes has resulted in a slightly atypical SRS phenotype. In contrast to current thinking, which favors the involvement of imprinted genes, we hypothesize that SRS may be caused by the inheritance of an additional copy of chromosome 7 material, either as a result of small duplications or undetected trisomy. Investigations of these possibilities may reveal the nature of the genetic abnormalities underlying this disorder.

Joyce CA, et al. *Hum Genet* 1999;105:273-280.

Editor's comment: *SRS is a very common cause of intrauterine growth retardation and subsequent short stature. Its etiology is undoubtedly heterogeneous and is beginning to be unraveled. This article contributes significantly to that task.*

Judith G. Hall, OC, MD

Please Send Correspondence to:
Robert M. Blizzard, MD
University of Virginia, The Blake Center
1224 West Main Street, 7th Floor, Suite 701
Charlottesville, VA 22903

Growth in Sotos' Syndrome

Persons with Sotos' syndrome have early accelerated growth, advanced bone age (BA), acromegaloid features, and developmental delay. Typically, the facies is distinguished by frontal bossing, large head circumference, antimongoloid slant of the palpebral fissures, and a prominent jaw. Diagnosis is based on the typical facies together with the large body size for age. Agwu et al report growth data on 40 patients (20 males and 20 females) who achieved their adult height. In addition to measurements of each patient's height and weight, arm span and body segment ratios were determined. Age of menarche was recorded, BA was determined, and target heights were calculated.

In boys, the mean height SDS in the 2nd and 6th years of life was 3.58 and 3.0, respectively. The mean height SDS for girls was 3.6 and 3.8 in the 2nd and 6th years, respectively. However, the mean height SDS at final height was 1.51 for men and 1.8 for women. These final heights are within the normal range for the British population for women and usually in the normal range for the men. However, the average final height in the men was 11.3 cm greater than their target height, whereas the average final height for women was only 6.2 cm above their target height. Upper to lower body segment ratio was reduced (Figure) and arm span was increased compared with population controls. BAs were advanced above the 97th percentile in those cases for which BAs were available. The mean age of menarche was 12.2 years (range, 8.9 to 15.4 years), which is slightly, but not significantly, earlier than the average for British girls (13 years). The excess arm span and reduced upper to lower body segment ratios suggest that much of the influence on height is a result of increases in limb lengths.

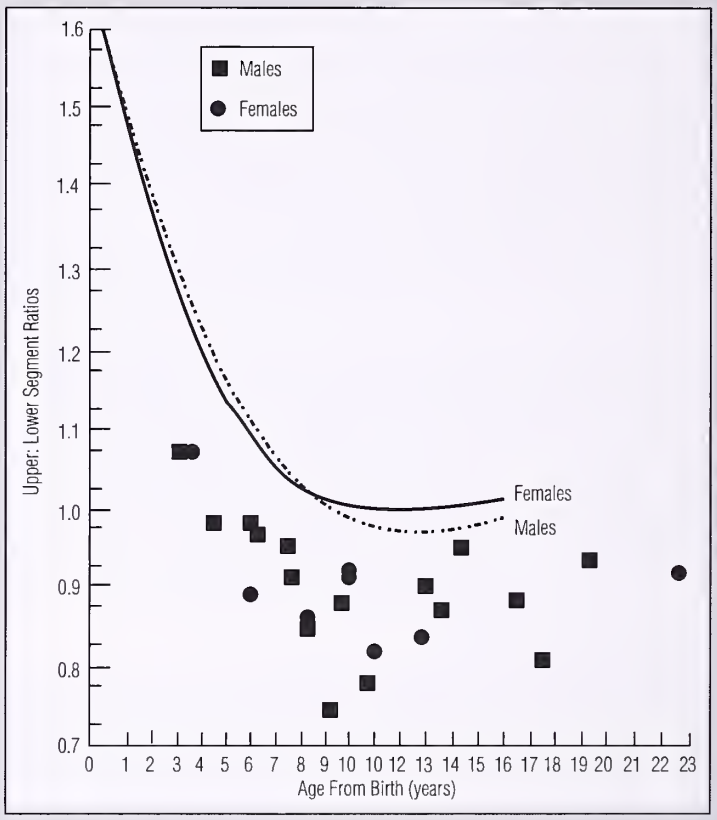
Agwu J, et al. *Arch Dis Child* 1999;80:339-342.

Editor's comment: *There has been significant concern as to whether linear growth velocity should be reduced in individuals with Sotos' syndrome. Agwu et al demonstrate that the final height of these individuals is not excessive even though it is somewhat above their target height. The article, which presents interesting and important information, would have been strengthened by the inclusion of separate growth curves for girls and boys in the study. Such graphic display of growth velocity at different times during childhood would have enhanced the readers' ability to understand the data. It appears that children with Sotos' syndrome are born large and remain large throughout infancy and childhood, enter puberty slightly earlier than the normal population, and achieve their final height within the population norm. Thus, the information should be useful to physicians caring for these individuals. Steroid intervention must be individualized since the mean adult heights fall within the normal range, although patients may be tall for their target heights.*

William L. Clarke, MD

Figure

Upper to Lower Body Segment Ratio in Patients With Sotos' Syndrome Compared With Normal Values for Age



Reprinted with permission from Agwu J, et al. *Arch Dis Child* 1999;80:339-342.

Growth Hormone Treatment in Young Children With Down's Syndrome: Effects on Growth and Psychomotor Development

Between the ages of 6 months and 3 years, children with Down syndrome experience a significant reduction in growth velocity, and it also is during that time that a decline in intelligence quotient (IQ) is noted. Thus, Annerén et al treated 15 children (6 boys and 9 girls) with Down syndrome with exogenous GH (0.1 IU/k/d) for 3 years beginning at 6 to 9 months of age. Height, weight, and head circumference were measured every third month during the first year, every 6 months during the second and third years, and 12 months after therapy. In addition, tests of motor development (motor perceptual tests) and mental development (Griffith's test) were performed before GH treatment, 1 year into treatment, at the end of treatment, and 1 year after treatment was stopped. Measurements were made of insulin-like growth factor 1 (IGF-1) and serum IGF-binding protein 3 (IGFBP-3). Fifteen aged-matched children with Down syndrome served as controls. No child in either the treated or control group had any cardiac malformations.

Two girls dropped out of the GH treatment group during the first year: one because of the development of celiac disease and the other because of an increase in serum aminotransferases. There

were no noted side effects from GH therapy. Specifically, frequent complete blood cell counts were performed to monitor for the risk of acute lymphoblastic leukemia, which is known to occur more frequently in children with Down syndrome. Prior to treatment, the study group had a height SDS of –1.8 and the control group had a height SDS of –1.7. After 36 months of treatment, the study group SDS rose to –0.8, whereas the control group SDS decreased to –2.2 (Figure). The study and control groups both had better growth than the average child with Down syndrome. The mean IGF-1 SDS in the study group was –1.6 before treatment, 0.28 after 3 years of treatment, and –1.11 one year after the end of treatment. There was a significant difference in head circumference between the 2 groups at the start of therapy, but there was no significant change in head circumference SDS in either group throughout the treatment period. At the end of GH treatment, the mean height of the treated group was significantly above that of the control group, but during the years after treatment these children grew less than the control group. However, there was a slight difference in heights at the age of 6.5 years (3 years after the end of treatment; $P < 0.05$). The mean IQ decreased in the GH-treated group, and no difference between the treated and untreated groups was noted at the age of 3½. These results demonstrate that GH can increase height velocity in children with Down syndrome but has no effect on head circumference or mental performance.

Annerén G, et al. *Arch Dis Child* 1999;80:334-338.

Editor's comment: *This carefully conducted study provides significant data useful for making decisions about whether to treat children with Down syndrome with GH. Interestingly, the authors concluded that they would not recommend GH treatment of such children unless GH deficiency was proven. Indeed, there was no effect on mental or motor development, and the differences in height at 3 years posttreatment were minimal. Whether such short-term effects of GH in children with Down syndrome would be observed had there been cardiac or other significant congenital malformations remains to be shown. It would appear that should GH treatment be used, it may be necessary to continue therapy until adult height is achieved.*

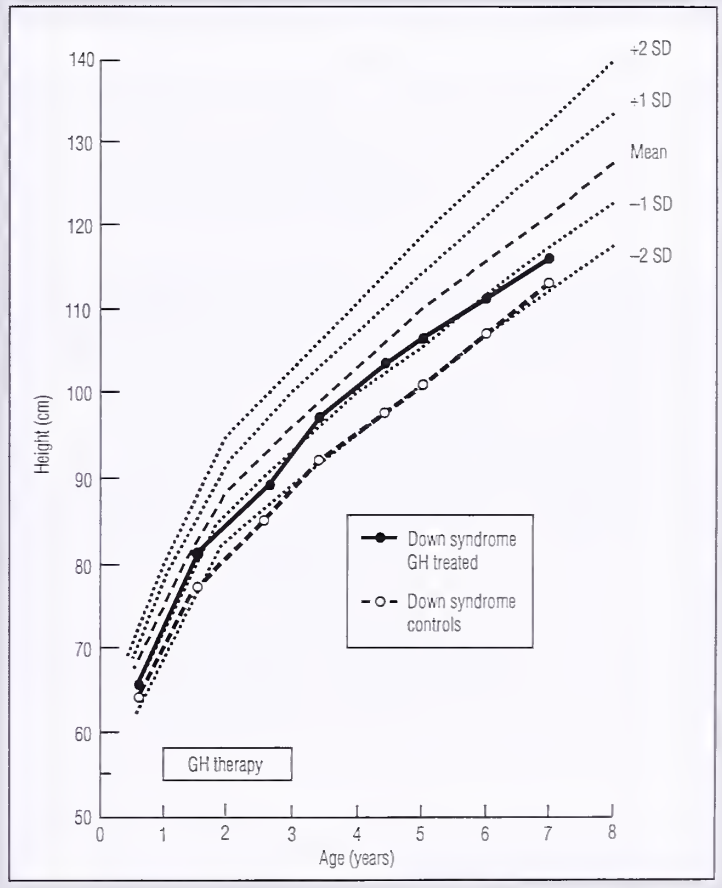
William L. Clarke, MD

2nd Editor's comment: *These data demonstrate that administration of GH therapy to infants with trisomy 21 does not prevent the deterioration in mental development that occurs in patients with Down syndrome between 12 and 36 months of age (ie, IQ scores declining from 70 to 40). The effect of GH therapy on the fine motor skills of these children requires validation and assessment of its significance and impact on the quality of life of these subjects. At present, routine administration of GH therapy to children with trisomy 21 cannot be recommended outside of controlled investigative studies.*

Allen W. Root, MD

Figure

Mean Height of 12 Boys and 3 Girls With Down Syndrome Treated With GH for 3 Years From the Age of 6 to 9 Months (Mean, 7.4 Months), Compared With That of an Untreated Group of 6 Boys and 9 Girls With Down Syndrome



The results are presented on the Swedish growth chart for normal boys.

Reprinted with permission from Annerén G, et al. *Arch Dis Child* 1999;80:334-338.

Growth Hormone Improves Body Composition, Fat Utilization, Physical Strength and Agility, and Growth in Prader-Willi Syndrome: A Controlled Study

Fifty-four children, aged 4 to 16 years, with genetically confirmed Prader-Willi syndrome (PWS) were enrolled in this randomized, placebo-controlled study using GH. Subjects continued to pursue habitual energy intake throughout the study, and their diet history was analyzed every 6 months. After a 6-month period, children were randomly assigned to receive either placebo (n=19) or 1 mg/m²/d of GH therapy (n=35) for 12 months. Stimulated GH levels (with clonidine) and serum levels of insulin-like growth factor 1 (IGF-1), IGF-binding protein 3 (IGFBP-3), osteocalcin, type 1 procollagen, fasting and 2-hour glucose and insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, free fatty acid, triglycerides, free thyroxine (T_4) and thyroid-stimulating hormone (TSH) were determined at 0 and 12 months. Height was measured at – 6, 0, 6, and 12 months. The mean baseline growth rate of all children was 4.8 ± 1.7 cm/y. Bone age was determined annually. Body fat (BF), lean body mass (LBM), and bone mineral density

(BMD) were determined by dual energy X-ray absorptiometry (DXA). Resting energy expenditures (REE) and respiratory quotients (RQ) were determined by indirect calorimetry. Physical strength and agility were determined by using a modified Bruininks-Oseretsky test.

After 12 months, GH-treated children had a mean growth rate of 10.1 cm/y, compared with 5 cm/y among controls. Mean IGF-1, IGFBP-3, osteocalcin, and type 1 procollagen levels significantly increased in GH-treated children. Baseline body composition analysis revealed increased BF (45.2% ± 8.3% vs 18% ± 3.6%) and lower LBM (50% vs 80%) compared with healthy children without PWS. GH-treated patients experienced an 8% decrease in BF. Their LBM increased compared with controls (Table). Ninety-five percent of PWS children had normal baseline BMD. Femoral BMD increased by 0.09 ± 0.02 g/cm^2, while lumbar spine and total body BMD remained unchanged during GH treatment. Although baseline REE was low in PWS, it did not increase after GH treatment; however, RQ values decreased during GH treatment. GH treatment increased strength, agility, and respiratory muscle force. GH therapy improved the lipid profile as cholesterol and HDL cholesterol levels fell. Lastly, GH therapy did not significantly affect fasting and 2-hour postprandial insulin levels.

The authors concluded that GH treatment promoted growth and positively affected body composition, physical strength, and agility in children with PWS.

Carrell A, et al. *J Pediatr* 1999;134:215-221.

Editor's comment: *Obesity, short stature, and hypotonia are some of the most striking physical signs of children with PWS. This interesting, well-designed, prospective study describes the effects of GH treatment—promoting growth while improving body composition and physical capacity—in children with PWS. The data are in agreement with previous studies and clearly showed that GH therapy could represent one of the most important therapeutic approaches to this disorder.*

The response of PWS patients to GH treatment resembles the positive effects of GH in hypopituitary patients. In addition, the possibility that PWS patients are GH deficient because of hypothalamic abnormalities needs to be considered, although the GH axis has been difficult to assess because of the obesity of PWS patients. The deleterious effects of GH treatment should be considered as PWS children have an increased incidence of scoliosis, which could be aggravated by GH treatment. Finally, the chromosomal abnormalities characteristic of PWS may add a risk factor for potential GH-associated malignancies. Therefore, the estimated potential benefit of this kind of treatment should be evaluated on a long-term basis.

Fima Lifshitz, MD

2nd Editor's comment: *This topic is of much current interest. To supplement your knowledge about the recent literature, you can refer to 2 articles abstracted in* GGH *previously (1999;15[1]).*

1. *Thacker MJ, et al. Growth failure in Prader-Willi syndrome is secondary to GH deficiency.* Horm Res *1998;49:216-220.*
2. *Lindgren AC, et al. Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favorably.* Acta Paediatr *1998;87:28-31.*

The data and conclusions in these articles are consistent.

Robert M. Blizzard, MD

Table
Body Composition, Bone Mineral Density, and Energy Expenditure

	Baseline		12-Month Values	
	Control Group (n=19)	GH Therapy Group (n=35)	Control Group (n=19)	GH Therapy Group (n=35)
Body fat (%)	42.6 ± 8.1	46.3 ± 8.4	45.8 ± 8.8	38.4 ± 10.7*
Lean mass (kg)	20.5 ± 5.0	20.5 ± 6.3	21.7 ± 5.0	25.6 ± 4.3*
BMI (kg/m^2)	24.2 ± 6.5	25.0 ± 6.7	25.2 ± 8.9	23.7 ± 6.3
Femoral neck BMD (g/cm^3)	0.636 ± 0.09	0.656 ± 0.19	0.707 ± 0.09	0.797 ± 0.09†
Spine BMD (g/cm^3)	0.753 ± 0.12	0.744 ± 0.14	0.793 ± 0.13	0.834 ± 0.15
REE (kcal/m^2/h)	22.5 ± 3.4	22.4 ± 4.4	25.1 ± 6.9	28.2 ± 7.4
RQ	0.83 ± 0.1	0.81 ± 0.07	0.84 ± 0.04	0.77 ± 0.04*

BMI, body mass index; BMD, bone mineral density; REE, resting energy expenditure; RQ, respiratory quotients.
*$P < 0.01$ paired t test before and after GH therapy, compared with either baseline values of treated patients or 12-month values of nontreated patients.
† $P < 0.05$ paired t test before and after GH therapy, compared with either baseline values of treated patients or 12-month values of nontreated patients.

Reprinted with permission from Carrel A, et al. *J Pediatr* 1999;134:215-221

Growth Hormone Treatment Increases CO_2 Response, Ventilation and Central Inspiratory Drive in Children With Prader-Willi Syndrome

Hypoventilation commonly occurs in subjects with Prader-Willi syndrome (PWS). This has been attributed in large part to obesity and the imposition of a large mechanical load, thereby impairing movement of the thoracic cage. However, there also is a problem with ventilatory control in PWS patients, as evidenced by abnormal responses to hyperoxia, hypoxia, and hypercarbia, as well as decreased peripheral chemoreceptor activity (Menendez AA. *Eur J Pediatr* 1999;158:941-942).

Lindgren et al studied resting ventilatory regulation in 9 prepubertal children with PWS prior to and after 6 to 9 months of therapy with rhGH (0.23 mg/kg/wk). This therapeutic program did not alter body mass index in this interval, although changes in body composition were not reported. They found that in response to rhGH, minute ventilation (mL/kg/min) increased 126%; the ventilatory response (ie, sensitivity) to 4% CO_2 increased 8-fold; and the central inspiratory drive, a measurement of airway occlusion pressure that is little affected by pulmonary mechanics (determined by the change in airway pressure during the first 0.1 second after beginning an inspiration), increased 170%. In contrast, the response to hyperoxia, measured as an increase of the inspired O_2 concentration from room air to 100%, did not change during rhGH administration.

The investigators suggest that the impairment of respiratory function in PWS may be due in large part to an abnormality in the function of a hypothalamic respiratory regulatory center rather than to excessive weight, that rhGH may have had a direct central stimulatory effect on this structure, and that the hypoventilation of PWS may be amenable to treatment.

Lindgren AC, et al. *Eur J Pediatr* 1999;158:936-940.

Editor's comment: *Although current data did not explore the possibility that the increased respiratory effort during rhGH treatment of PWS subjects was due, at least in part, to increase in lean body mass and improved muscle strength, these findings support the suggestion that rhGH may be useful in the management of PWS, which is a conclusion that this reviewer has been very reluctant to draw. Lindgren et al* (Acta Paediatr Scand 1998;87:28-31) *also have reported that in a controlled, randomized trial of rhGH in PWS subjects, rhGH led to increases in growth, lean body mass, physical activity, and endurance, and improvements in behavior. Carrel et al* (J Pediatr 1999;134:215-221) *confirmed these findings and also observed that rhGH improved physical strength, agility, and inspiratory and expiratory muscle strength in PWS. Since chronic respiratory insufficiency often leads to pulmonary hypertension and right ventricular heart failure, and is a major cause of death in PWS, treatment of PWS may be expanded to include diet, exercise, and rhGH. Before rhGH becomes the standard of care for PWS, however, further controlled and, hopefully, double-blind studies must be conducted that confirm these reports and justify the large expenditures that such treatment will engender, as well as demonstrate that the quality of life of rhGH-treated PWS subjects is superior to that achieved by a rigorous residential dietary and exercise program alone.*

Several abstracts and editorial comments regarding the treatment of PWS have appeared in GGH *in 1999 (see Vol 15, pages 10 and 11).*

Allen W. Root, MD

Postnatal Sex Reversal of the Ovaries in Mice Lacking Estrogen Receptors α and β

The investigators developed mice in which both estrogen receptors (ERs) α and β had been disrupted or "knocked out" (αβERKO) by breeding phenotypically normal mice heterozygous for loss of either one or the other ER. αβERKO mice were phenotypically intact and survived normally. Adult male mice in which both ERs had been eliminated had normal internal genitalia but slightly smaller testes than control animals, with loss of germinal epithelium and subnormal spermatogenesis; they were infertile, which is consistent with earlier reports of the αβERKO male—indicating that estrogen and the ER are necessary for complete spermatogenesis. Adult female mice in which both ERs had been disrupted also had normal but hypoplastic internal genitalia, indicating that neither ER is necessary for Müllerian duct differentiation. Having ERα is necessary for uterine response to estrogen. The ovaries of αβERKO adult female mice contained both healthy (with oocytes) and sex-reversed follicles; the latter were characterized by degeneration of the oocyte and "transdifferentiation" of follicles into seminiferous-like tubules, with Sertoli-like cells that expressed increased amounts of mRNA for *Sox9/MIS*. Similar changes are not observed in adult female mice who lack one or the other ER.

The authors suggest that the ovarian follicles of the αβERKO adult female mice "redifferentiated" into testes-like structures. Although sex reversal of fetal rodent ovaries has been accomplished by their transplantation into an adult animal or one in which there is transgenic overexpression of *MIS* or by in vitro exposure to *MIS*, it previously has not been recorded in adult ovaries. The investigators suggest that in the absence of both ERs, the differentiated ovarian follicle is able to form a testes-like structure, possibly because of the loss of estrogen-mediated persistent repression of *MIS* and *Sox9*.

Couse JF, et al. *Science* 1999;286:2328-2331.

Editor's comment: *This paper documents the need for both ER α and β and therefore estrogen to maintain ovarian differentiation in the adult mouse.* Wnt-4 *recently has been shown to be necessary for ovarian and Müllerian duct differentiation and* BAX *regulates the longevity of the ovarian follicle. Future studies will be directed to elucidating the mechanism(s) of estrogen action and the identification of other factors involved in this complex process. If the reader has not read the lead article in this issue of* GGH *titled "Estrogen and Growth", he/she will miss a golden opportunity to integrate these 2 presentations regarding the actions of estrogen. Keep in mind, however, that mice and humans may not be identical in all actions of estrogen.*

Allen W. Root, MD

Directed Pharmacological Therapy of Ambiguous Genitalia Due to Androgen Receptor Gene Mutation

The authors report a 46,XY infant with ambiguous genitalia and undescended gonads due to the partial androgen insensitivity syndrome (PAIS). A T→C transition resulted in a missense mutation of codon 807 in the ligand binding region of the androgen receptor (AR). The mutated AR had only 15% of the binding capacity for testosterone and 15% of the in vitro transcription activating function as the wild-type AR. Yet this mutated AR bound dihydrotestosterone (DHT) with high efficiency and effective function. Topical periscrotal application of a preparation of DHT gel led to raised serum DHT concentrations, rugation of the scrota, descent of the gonads, and enlargement of the phallus. The authors conclude that functional assays as described of a mutated AR may identify subjects who are androgen responsive and thus could be reared in their genetic sex with DHT administration.

Ong YC, et al. *Lancet* 1999;354:1444-1445.

Editor's comment: *These data demonstrate the direct clinical benefit that may accrue to selected subjects with a mutated AR if the mutated gene's structure and function can be evaluated rapidly after birth. The authors do not state how much time it took to complete the molecular studies. Nordenstrom et al* (J Clin Endocrinol Metab 1999;84:1505-1509) *suggested that genotyping of neonates with 21-hydroxylase-deficient congenital adrenal hyperplasia detected in neonatal screening programs will identify infants at significant risk for the salt-losing form of this disease and thus permit more specific management.*

The case report of Ong et al raises the question of why the genitalia of the reported infant was ambiguous if DHT was biologically effective in utero. It is clear that male fetuses with deficiency of 5α-reductase are not virilized in utero and, thus, DHT is essential for this process. The investigators demonstrated that the M870T AR did not respond well to lower levels of DHT, although the maximum responses of the mutated and wild-type receptors were similar. Thus, in utero physiologic levels of DHT must have been biologically ineffective. An excellent review of the mutations identified in the human AR patient has been published recently by McPhaul and Griffin (J Clin Endocrinol Metab 1999;84:3425-3441).

Allen W. Root, MD

Drosophila S6 Kinase: A Regulator of Cell Size

Although many of the growth factors and intracellular signaling pathways that regulate cellular multiplication have been deciphered, the mechanisms that determine cell size are not as widely understood. These investigators report that in *Drosophila melanogaster* inactivation of the gene *Drosophila* S6K = *dS6K*, which encodes a physiologic kinase for the ribosomal protein S6, inhibits increase in cell size without affecting the cell number of a given structure. The S6 kinases control the translation of mRNAs that in turn encode ribosomal proteins involved in the translational process. *dS6K* was inactivated by altering the 5′ noncoding or transcription-activating region of the gene, or by excising part of the first exon of the gene that encodes a portion of the catalytic domain.

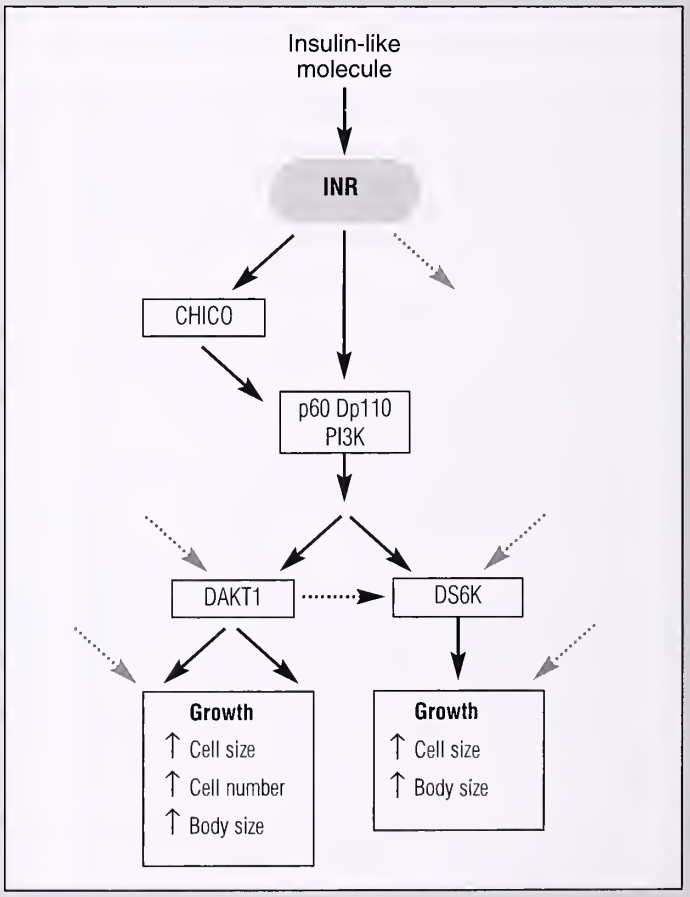
Insects with a homozygous null mutation in *dS6K* were symmetrically dwarfed to half the size of normal flies. Cell numbers in both the eyes and wings of the dwarfed insects were similar to those in control insects but the cell sizes were 30% smaller. Inhibition of cellular growth was present in the larval stage of insects homozygous for inactivation of *dS6K*. The investigators also generated insects that expressed 3 copies of *dS6K* in targeted segments; these insects apparently had larger cell sizes in selected structures. The authors conclude that the kinase encoded by *dS6K* regulates cell size without affecting cell number in *Drosophila melanogaster.*

Montagne J, et al. *Science* 1999;285:2126-2129.

Editor's comment: *Under normal circumstances, organ and body size reflect both cell number and cell size. The 2 processes of cell growth and replication seem to be coupled. Identification of a factor that regulates cell size specifically is an important advance in our understanding of the mechanisms that influence growth. In both insects and mammals, S6 kinases regulate synthesis of ribosomal proteins that are encoded by 5′-terminal oligopyrimidine tract mRNAs. These proteins influence translation (Leevers SJ.* Science *1999;285:2082-2083). S6 kinases are under the control of the insulin signaling pathway involving the insulin receptor, insulin receptor substrates, phosphatidylinositol 3-kinase (PI3K), and its target Akt/PKB or DAKT1 (Figure). Drosophilae with loss-of-function mutations in the insulin receptor substrate are small and cell size and number are both reduced, whereas loss of the insulin receptor, PI3K, or DAKT1 is lethal. The difference between these experimental models of impaired growth suggests the presence of alternate but interactive intracellular pathways regulating cell growth and cell division.* Drosophila *with loss-of-function mutations in other genes* (Minutes) *that regulate ribosomal protein synthesis also have slow rates of cell growth and replication but normal cell size. The application of these data to our patients with primordial disorders of growth seems imminent. To follow the resultant revelations will be exciting.*

Allen W. Root, MD

Figure
Signaling for Growth



The likely interactions (solid arrows) between molecules that regulate *Drosophila* growth, based on studies of interactions between their mammalian homologues. Broken arrows indicate where branching is possible and where links with other molecules may occur. INR, the *Drosophila* insulin receptor homologues; CHICO, the *Drosophila* homologue of mammalian IRS1-4; Dp 110, the *Drosophila* class 1^{A} PI3K; p60, its adaptor protein; and DAKT1, the *Drosophila* homologue of Akt/PKB.

Reprinted with permission from Montagne J, et al. *Science* 1999;285:2126-2129

GROWTH, Genetics, & Hormones Table of Contents for Volume 14, 1998 and Volume 15, 1999

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

Please note that each question may have more than one correct answer.

1. Which of the following beliefs in the past remain beliefs currently?
   a. Estradiol turns off GH secretion.
   b. Children produce more GH than young adults.
   c. Estradiol is primarily responsible for the adolescent growth spurt in females.

2. Estradiol 800 ng/kg/d has been demonstrated to ____ in patients with TS.
   a. Increase GH secretion.
   b. Increase IGF-1 levels.
   c. Inhibit ulnar growth.

3. Which of the following is/are true?
   a. In familial male precocious puberty, androgen antagonistic therapy is effective in slowing the growth rate and epiphyseal maturation.
   b. Estradiol increases the pulse amplitude and frequency of GH.

4. The phenotype of a pubertal-aged female with aromatase deficiency includes which of the following?
   a. Prominent adrenarche.
   b. Acne.
   c. Virilization.
   d. Advanced bone age.

5. The phenotype of estradiol deficiency in the male includes:
   a. Tall stature.
   b. Impaired epiphyseal maturation.
   c. Eunuchoid habitus.
   d. Acromegaloid features.

Answer Key: 1. c 2. a,b,c 3. a 4. a,b,c 5. a,b,c

CME Accreditation Statement

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

UNIVERSITY OF VIRGINIA HEALTH SCIENCES CENTER
SCHOOL OF MEDICINE

The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Disclosure: As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Frank, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Allen has received grant/research support from, and has served as a scientific advisor to, Genentech, Inc. Dr. Root serves on Genentech Inc.'s National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc. Drs. Allen and Fost received a grant from The Genentech Foundation to host the conference described in this issue.

GROWTH, Genetics, & Hormones is published under an unrestricted educational grant from Genentech, Inc.

Robert M. Blizzard, MD
c/o Gardiner-Caldwell SynerMed
405 Trimmer Road
PO Box 458
Califon, NJ 07830

GROWTH
Genetics & Hormones
Vol. 16 No. 2 July 2000

Effects of Glucocorticosteroids on Growth

Otto Mehls, MD, PhD
Professsor of Pediatrics
Burkhard Tönshoff, MD, PhD
Assistant Professor
Division of Pediatric Nephrology
University Children's Hospital
Heidelberg, Germany

INTRODUCTION

Glucocorticoid therapy in pharmacologic doses is the treatment of choice in various chronic inflammatory and immune-mediated diseases in childhood. Glucocorticoids also are needed for immunosuppression after organ transplantation. Long-term, high-dose glucocorticoid treatment inevitably leads to protein catabolism and growth failure. Recent evidence indicates that these side effects are partially mediated by alterations of the somatotropic hormone or growth hormone (GH) axis.

This review summarizes our current knowledge of the pathogenesis of glucocorticoid-induced growth failure. It also summarizes available therapeutic options. In particular, it answers the question whether glucocorticoid-induced growth failure can be counterbalanced by concomitant treatment with recombinant human GH (rhGH).

PATHOGENESIS OF GLUCOCORTICOID-INDUCED GROWTH FAILURE

Effects on GH Secretion

While short-term glucocorticoid administration stimulates GH secretion,[1,2] long-term, high-dose treatment diminishes spontaneous GH secretion (Table 1). Several studies performed in man[3] have demonstrated that the inhibitory effect of these steroids on GH secretion in vivo are due to enhancement of hypothalamic somatostatin (SRIF) release. Recent data indicate that arginine infusion is able to normalize the GH response to GH-releasing hormone by inhibition of the endogenous hypothalamic SRIF tone.[4] In peripubertal children with renal transplants, a significant reverse relationship between the daily dose of glucocorticoids and the peak amplitude or mean levels of GH was noted, whereas the GH pulse frequency was not changed.[5] Similar results were seen in experimental rats treated with methylprednisolone.[6]

Table 1
Interference of Long-Term, High-Dose Glucocorticoid Treatment With the Integrity of the Somatotropic Hormone Axis

Organ	Effect of Glucocorticoids
Hypothalamus	Somatostatin tone ↑
Hypophysis	GH secretion ↓
Liver	GH-induced IGF-1 mRNA ↓
Circulation	IGF-1 levels normal or ↑ Induction of IGF-1 inhibitors IGFBP-2 ↑
Epiphyseal growth plate	Cell proliferation ↓ Matrix production ↓ Paracrine IGF-1 secretion ↓ GH and type 1 IGF receptor ↓

In This Issue

This educational activity is supported by an unrestricted educational grant from Genentech, Inc.

Effects on GH Receptor Expression

There is experimental evidence that these steroids suppress GH receptor expression, at least in the liver. While adrenalectomy does not appear to affect hepatic GH receptor mRNA and binding or plasma levels of GH-binding proteins (GHBPs) in the rat, these parameters were markedly reduced in a dose-dependent fashion by dexamethasone (DEX) treatment.[7] Estrogens[8] as well as glucose and amino acids[9] interact with DEX to control the expression of GH mRNA in cultured hepatocytes. Under clinical conditions, GH receptor status can be assessed by determination of the high-affinity GHBP, which is derived from the extracellular domain of the GH receptor by proteolytic cleavage. We observed a significant reduction of circulating GHBP levels compared with age-matched controls.[10]

Effects on Insulin-Like Growth Factors and Insulin-Like Growth Factor-Binding Proteins

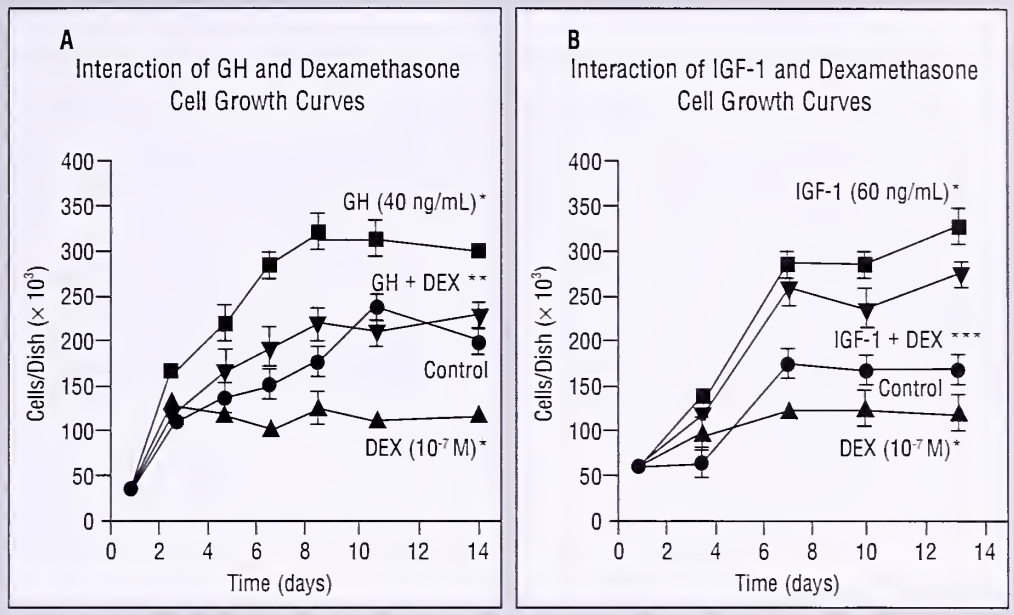
Secondary to impaired GH secretion and GH receptor expression by glucocorticoids, one would expect a decrease of circulating insulin-like growth factor (IGF). However, controversial findings have been reported so far. Under experimental conditions, the GH-induced rise in serum IGF-1 in *hypophysectomized* rats was significantly inhibited by high doses of DEX.[11] Concomitantly, the GH-induced IGF-1 mRNA content in the liver and various other tissues was inhibited by pretreatment with DEX. There also was a reduction in the hepatic IGF-1 mRNA in DEX-treated *intact* rats, which, however, did not result in decreased IGF-1 serum levels. The authors suggested 2 possible explanations: (1) Hepatic IGF-1 mRNA is not translated into protein, or (2) glucocorticoids alter IGF-1 translation, synthesis, and secretion in such a way that IGF-1 mRNA no longer reflects IGF protein synthesis. In patients with chronic endogenous glucocorticoid excess, IGF-1 levels were elevated.[12]

Although glucocorticoids do not consistently reduce circulating immunoreactive IGF-1 levels, they inhibit IGF bioactivity both in children with a variety of disorders, including the nephrotic syndrome,[13] and in adults given a single 16-mg dose of prednisone.[14] Hence, glucocorticoid excess apparently does not impair immunoreactive IGF-1 levels, but rather antagonizes the action of IGF by direct and/or indirect mechanisms, possibly by increased production of IGF inhibitors. IGF inhibitors, which have a molecular weight of 12 to 20 kd and which clearly differ from IGF-binding proteins (IGFBPs), have been identified. In one study, glucocorticoid excess in patients with Cushing's syndrome was associated with a slight increase of IGFBP-3, normal IGFBP-1 levels, and clearly elevated IGFBP-2 levels.[12] In contrast, DEX 5 mg given for 4 days to normal volunteers decreased IGFBP-2 levels while nearly doubling serum IGF-1 concentrations, which was in parallel to an increase of serum IGFBP-3 levels.[15] Further studies to explain these apparent paradoxes are needed.

Effects of Glucocorticoids on Growth Plate Chondrocytes

These steroids inhibit sulfation of cartilage matrix, mineralization and formation of new bone, and cell proliferation in the growth zone. Contrary to expectations, DEX caused a tissue-specific stimulation of GH receptor mRNA associated with a biphasic dose-response relationship in rabbits.[16] These data suggest that glucocorticoid-induced GH insensitivity cannot be explained by decreased GH receptor mRNA levels. To the contrary, DEX causes a tissue-specific stimulation of GH receptor mRNA levels with a biphasic dose-response relationship. In contrast, investigators using cell culture experiments demonstrated that DEX time dependently decreased DNA synthesis and cell proliferation of epiphyseal chondrocytes through a reduction of GH receptor expression and inhibition of homologous upregulation of both the GH and

Figure 1



Effect of DEX, GH, and IGF-1 on chondrocyte proliferation in primary cultures (growth curves). The cells were synchronized (starved), and the medium was then changed to one containing 0.2% bovine serum albumin and 0.3% Ultroser plus hormones and solvent as indicated. Data are mean ± SE of 4 parellel dishes per group and day.

*P <0.001 vs control
**P <0.001 vs DEX and GH
***P <0.001 v. control, DEX and IFG-1 alone

Reprinted with permission from Jux C, et al. *Endocrinology* 1998;139:3296-3305.

IGF-1 receptor and through the inhibition of paracrine IGF-1 secretion (Figures 1 and 2).[17] These experiments have been confirmed in primary cultured hepatocytes.[18] Unfortunately, little is known about the precise mechanisms by which glucocorticoids regulate the gene activity that eventually leads to growth disturbance.

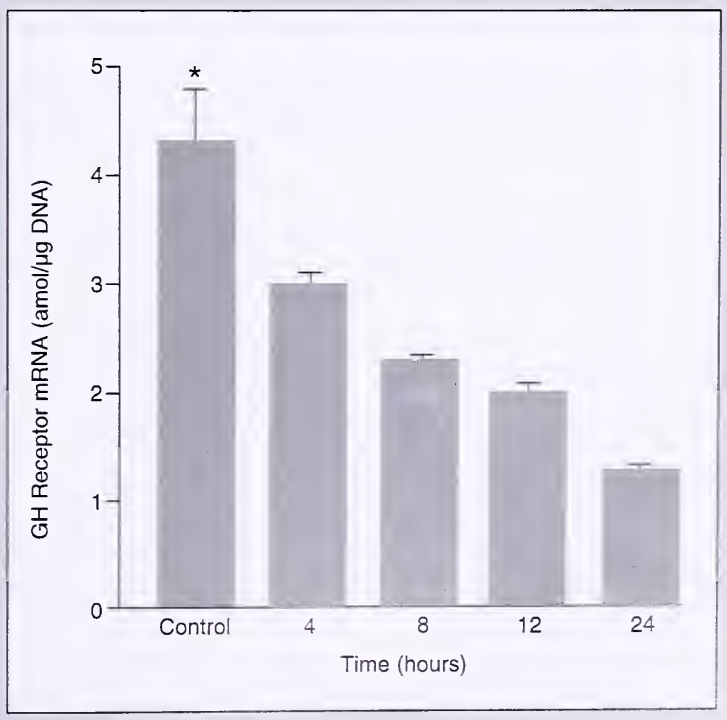
Glucocorticoids exert their genomic effects after cytosolic binding to specific receptors. Once conformational changes and translocation to the nucleus have occurred, the complexes bind to DNA at specific consensus sites, termed "glucoid response elements," on the upstream promoter sequence of steroid-responsive genes.[19]

Clinical Presentation

Only long-term systemic glucocorticoid treatment impairs growth, whereas inhaled or topical glucocorticoids do not significantly reduce growth in most patients. This general statement holds true for patients with bronchial asthma and atopic eczema.[20,21] In patients with either frequently relapsing or steroid-dependent nephrotic syndrome, growth-retarding effects up to temporary growth arrest have been observed. Those patients receiving repeated courses of high-dose steroids or prolonged maintenance therapy were at greatest risk of growth failure. However, when Foote et al[22] examined the heights of 80 patients with frequently relapsing nephrotic syndrome, it became evident that final height was only slightly reduced by −0.2 SD (equivalent to a height deficit of 1.5 cm below average height). Total glucocorticoid dose correlated negatively but only weakly with changes in height SDS.

Persistent hypoalbuminemia seems to be an independent risk factor for growth retardation, as seen in patients with steroid-resistant nephrotic syndrome.[23] In patients with juvenile chronic arthritis or inflammatory bowel disease, glucocorticoid therapy frequently is needed when nonsteroidal drugs fail to control symptoms. These patients frequently have growth retardation. However, the pathogenesis of growth failure in such patients is multifactorial. In chronic inflammatory disease, malnutrition and other factors are contributive and the relative contribution of glucocorticoids is difficult to assess, since systemic treatment usually is needed in the most severe cases. Inflammation

Figure 2



Time-dependent downregulation of GH receptor mRNA by DEX (10^{-7} M). RNase-protection solution-hybridization assay. *P<0.005 vs all treatment groups.

Reprinted with permission from Jux C, et al. *Endocrinology* 1998;139:3296-3305.

might be so active that growth is possible only with glucocorticoid treatment. Depending on the dose of steroids, the same treatment may permit or inhibit the growth rate.

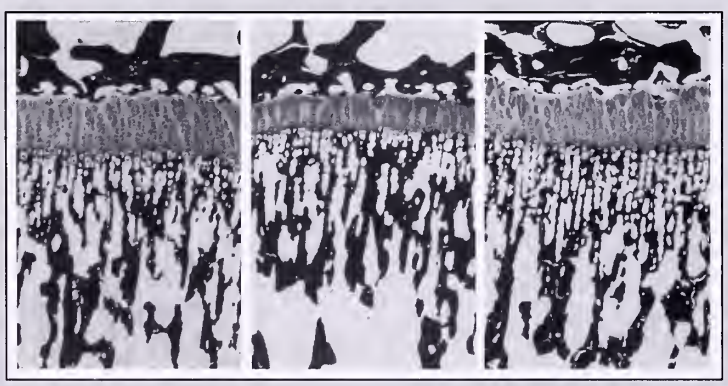
The situation seems to be more clear in pediatric transplant recipients, in whom glucocorticoid treatment is used for prevention of rejection episodes. According to the North American Pediatric Renal Transplant Cooperative Study, catch-up growth after renal transplantation is unlikely to occur in 75% of renal allograft recipients. It primarily occurs in recipients <6 years of age. Reduced allograft function and glucocorticoid treatment were identified as the main risk factors, and a negative correlation between growth and cumulative glucocorticoid dose is noted after correction for renal function.[24]

THERAPEUTIC OPTIONS

Intermittent Corticosteroid Treatment

There is general agreement that alternate-day treatment given in 1 dose significantly improves but does not normalize growth rates. Increased growth rates have been observed in patients with juvenile rheumatoid arthritis[25] and in patients with renal allografts.[26] Alternate-day treatment often is combined with a dose reduction of glucocorticoids. It has not been established to what extent the dose reduction or the intermittent mode of treatment contributes to the improvement in growth. The success of the treatment strategy varies from patient to patient. Therefore, catch-up growth does not consistently occur. One major problem is the individual's sensitivity to glucocorticoids, which currently cannot be satisfactorily measured.[27,28] Another problem is that only stable patients are shifted from daily to intermittent treatment, which skews the results. Clinical studies suggest that third-generation glucocorticoids such as deflazacort have fewer side effects while maintaining equipotent anti-inflammatory and immunosuppressive activity. Although uncontrolled clinical studies have demonstrated positive results,[29,30] controlled studies are missing, and no gold standard is available with which to compare the immunosuppressive properties of different glucocorticoids.

Figure 3



Light photomicrographs of proximal tibia growth plate. Sections stained with toluidine blue. Sham-operated control animals were fed ad libitum receiving either solvent (A), methylprednisolone (B), or methylprednisolone and GH (C). Methylprednisolone-induced growth retardation can be prevented by concomitant GH treatment.

Reprinted with permission from Kovacs G, et al. *Kidney Int* 1991;40:1032-1040.

Treatment With rhGH

During recent years, concomitant GH treatment has been demonstrated to diminish or even completely counterbalance the growth-depressing effects of glucocorticoid treatment. The success of such a treatment strategy seems to be dependent on the given dose, each individual's sensitivity, and the underlying primary disease. Furthermore, it is very likely that certain threshold doses, which may be different for different individuals, cannot be counterbalanced by rhGH. However, these doses are difficult to define.

Animal Studies

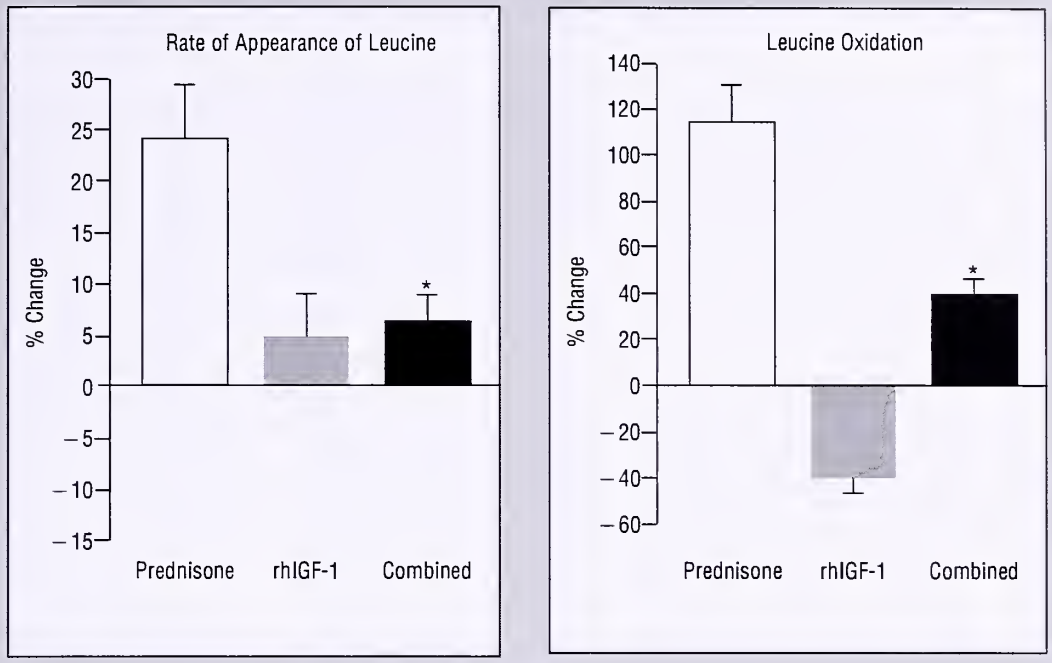
In cell culture studies focusing on growth plate chondrocytes, rhGH counterbalanced the antiproliferative effects of DEX in a dose-dependent manner.[17] In healthy and uremic female rats, rhGH was able to counterbalance prednisone in doses up to 9 mg/kg/d (Figure 3).[6] These results were extended by studies in rats demonstrating that administration of GH in vivo resulted in an increased cortical bone mass of both the vertebrae and the femoral diaphyses.[31] In piglets, DEX treatment resulted in lower plasma osteocalcin, urinary N-telopeptide, and whole-body and femoral mineral density. However, all of these could be prevented by concomitant treatment with GH.[32] In rat experiments from our laboratory,[6] methylprednisolone 2 mg/kg/d decreased and rhGH 0.6 mg/kg/d independently increased weight gain, whereas normal weight gain was observed with concomitant treatment.

Chrysis and Underwood[33] did not see a downregulation of the ubiquitin system in skeletal muscle with rhGH 3 mg/kg/d in catabolic rats receiving DEX 5 mg/kg/d, whereas downregulation was noted with IGF-1 treatment. This is consistent with the notion that GH affects protein synthesis but not proteolysis.

Studies in Man

Horber and Haymond[34] demonstrated that rhGH 0.1 mg/kg/d prevented the protein catabolic side effects of prednisone 0.8 mg/kg/d in 32 healthy adult volunteers. An extension of these studies by Bennet and Haymond[35] demonstrated that the anabolic effects were observed in subjects receiving long-term treatment with one quarter of the dose of glucocorticoids and one eighth of the dose of GH. Leucine kinetic data showed that the negative protein balance during prednisone treatment was due to increased proteolysis, whereas GH had no effect on proteolysis but increased whole-body protein synthesis. As

Figure 4



In healthy volunteers, oral prednisone (60 mg/d) increased both leucine turnover and leucine oxidation. The relative increase in protein turnover and oxidation was significantly decreased with concomitant rhIGF-1 treatment (100 µg/kg subcutaneously twice daily).

* $P<0.01$ vs prednisone-treated group

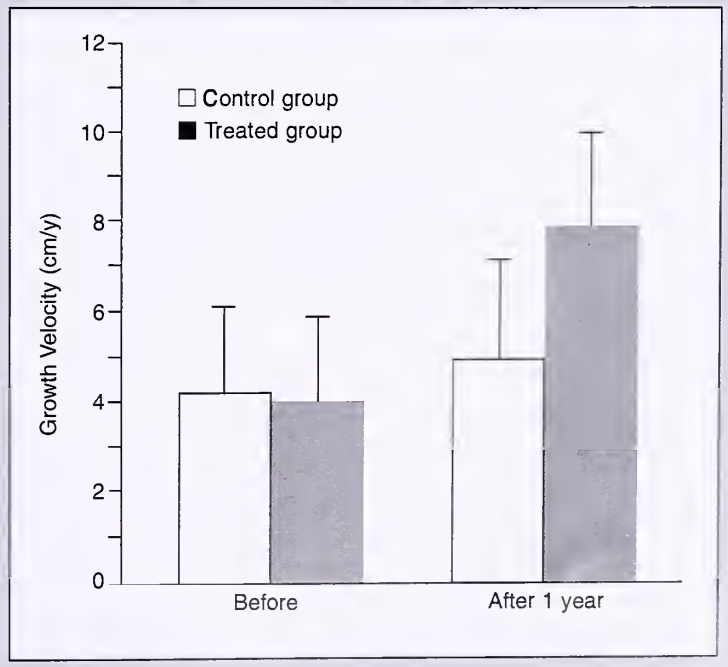
Reprinted with permission from Mauras N, Haymond MW. *Pediatr Nephrol* 1996;10:318-323.

prednisone and GH had differential effects on fuel metabolism and insulin antagonism, the authors assumed independent mechanisms were involved in which GH and prednisone may reciprocally regulate the oxidation of protein and fat while decreasing the efficiency of glucose disposal. Mauras and Haymond[36] examined the question of whether similar anabolic effects can be obtained with IGF-1 in prednisone-treated subjects without a reduction in carbohydrate tolerance. Like GH, IGF-1 in low doses increases protein synthesis, which is in contrast to the marked suppression of proteolysis seen with high doses. It also ameliorates the steroid-induced protein catabolism with no rise in plasma glucose and with a significant reduction in circulating insulin levels (Figure 4).

In early studies, no significant metabolic response to exogenous GH in glucocorticoid-treated children was noted.[37] In contrast, Davies et al[38] subsequently reported positive effects of rhGH on growth in growth-retarded children with rheumatoid arthritis. In a recent study, rhGH 1.4 IU/kg (0.5 mg/kg) per week was administered to 14 patients with rheumatoid arthritis. All patients showed an increase in growth velocity with a mean increase from 1.9 to 4.5 cm/y. This effect of pharmacologic doses of GH prevented a further decrease in height SDS, but it did not result in catch-up growth. All patients were severely stunted (mean height SDS, –4), which was the consequence of the primary disease and to a minor extent of glucocorticoid treatment.[39]

Allen and Goldberg[40] studied the effects of GH treatment (0.3 mg/kg/wk for 6 to 21 months) in 7 slowly growing children with various diseases treated with glucocorticoids. The mean height velocity increased from 3.4 to 6.7 cm/y. In the National Cooperative Growth Study,[41] 83 patients with various diseases who were receiving glucocorticoids and concomitant rhGH were identified. The mean height SDS was –3.7 ± 1.2 SD and the mean growth velocity was 3.0 ± 2.5 cm/y. After 12 months of rhGH therapy, the mean growth rate increased to 6.3 ± 2.6 cm/y and the mean height SDS improved by 0.21 ± 0.4 cm/y (P <0.01). Responsiveness to rhGH was negatively correlated with the dose of glucocorticoids (Figure 5).

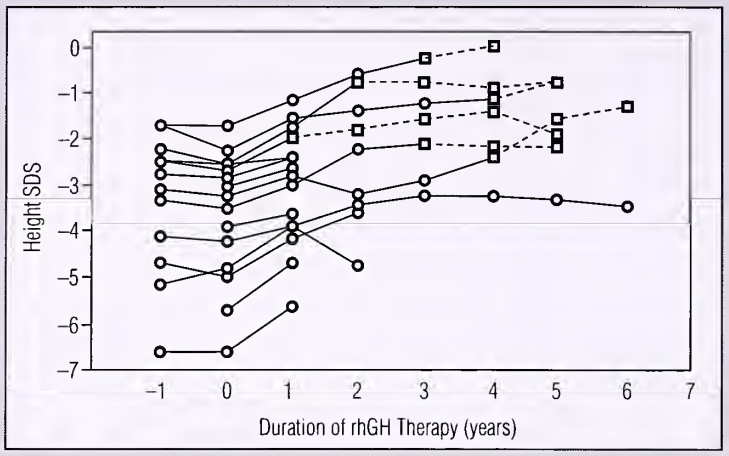
Figure 5



Growth velocity after 1 year of rhGH treatment in 85 children with a renal transplant and glucocorticoid treatment. The patients were randomized for rhGH treatment and controls. rhGH significantly increased growth velocity.

Reprinted with permission from Guest G, et al. *Pediatr Nephrol* 1999 12:437-446

Figure 6



Height SDS related to chronologic age in 14 children before and during 1 to 6 years of combined treatment with methylprednisolone and rhGH. Patients who entered puberty are indicated by ---□--- (O.M. and B.T., personal observations).

In glucocorticoid-treated children with renal allografts, the positive effect of GH treatment has been documented in a number of prospective, open-labeled studies (Figure 6).[42,43] Height velocity during the first treatment year in these children *prepubertally* can often be doubled by pharmacologic doses of rhGH. Promising results also have been obtained in growth-retarded *pubertal* children posttransplantation. Eighteen adolescents demonstrated an impressive growth response to rhGH. After 2 years of rhGH, they achieved an increase in height with a mean of 15.7 ± 5.1 cm, compared with 5.8 ± 3.4 cm in retrospectively matched controls.[44] This growth response occurred whether rhGH 4 or 8 IU (1.3 or 2.6 mg/m^2/d) was given. In a large randomized study involving 90 children treated with rhGH 30 IU (10 mg/m^2/wk), growth velocity was significantly increased from 4.1 to 7.7 cm/y; in the control group, no significant increase (4.2 to 4.6 cm) was noted. Four independent factors predicted the response to therapy: (1) slow growth velocity prior to GH treatment (negative); (2) low glomerular filtration rate (negative); (3) the mode of corticosteroid administration; and (4) a significant degree of insulin resistance (negative). However, overall the height velocity remained above baseline during 4 years of observations, and the mean height SDS increased from –3.5 to –2.5 within 3 years of treatment.[45]

The risk of GH treatment in children with renal transplantation must be considered with respect to possible deleterious effects on the survival of the transplant. Theoretically, there is concern that the immunostimulatory effects of GH might reduce the effectiveness of immunosuppression by glucocorticoids. Although *preliminary data* of the French randomized study showed a 35% increase in the rejection rate in high-risk patients who already had experienced more than 1 rejection episode before initiation of GH therapy,[46] in the *final analysis*,[47] biopsy-proven acute rejection episodes *were not* significantly more frequent in the group of patients who received rhGH. During the first year, 9 rejection episodes occurred in 44 rhGH-treated patients and 4 occurred in 46 control patients. Nevertheless, in all future studies in which rhGH is given concomitantly with glucocorticoids, careful analysis must be done to assess the potential negative effects of rhGH on immunosuppression.

REFERENCES

1. Casanueva FF, et al. *Neuroendocrinology* 1990;47:46-49.
2. Veldhuis JD, et al. *J Clin Endocrinol Metab* 1992;74:96-102.
3. Wehrenberg WB, et al. *Endocrinology* 1990;126:3200-3203.
4. Giustina A, et al. *J Clin Endocrinol Metab* 1992;74:1301-1305.
5. Schaefer F, et al. *J Pediatr* 1991;119:568-577.
6. Kovacs G, et al. *Kidney Int* 1991;40:1032-1040.
7. Gabrielsson BG, et al. *Endocrinology* 1995;133:2445-2452.
8. Bennett PA, et al. *Endocrinology* 1996;137:3891-3896.
9. Brameld JM, et al. *J Nutr* 1999;129:1298-1306.
10. Tönshoff B, Mehls O. In: Tejani AH, Fine RN, eds. *Pediatric Renal Transplantation.* New York, NY:John Wiley & Sons 1994:441-459.
11. Luo J, Murphy LJ. *Endocrinology* 1989;125:165-171.
12. Bang P, et al. *Acta Endocrinol* 1993;128:397-404.
13. Elders MJ, et al. *AJDC* 1975;129:1393-1396.
14. Unterman TG, et al. *J Clin Endocrinol Metab* 1985;61:618-626.
15. Miell JP, et al. *J Endocrinol* 1993;23:45-49.
16. Heinrichs C, et al. *Endocrinology* 1994;135:1113-1118.
17. Jux C, et al. *Endocrinology* 1998;139:3296-3305.
18. Beauloye V, et al. *Growth Horm IGF Res* 1999;9:205-211.
19. King APJ, Carter-Su C. *Endocrinology* 1995;136:4796-4803.
20. Allen DB, et al. *Allergy Clin Immunol* 1994;93:967-976.
21. Massarano AA, et al. *Arch Dis Child* 1993;68:677-679.
22. Foote KD, et al. *Lancet* 1985;11:917-919.
23. Schärer K, et al. *Pediatr Nephrol* 1999;13:828-834.
24. Fine RN. *Pediatr Transplant* 1997;1:85-89.
25. Byron MA, et al. *J Royal Soc Med* 1983;76:452-457.
26. Broyer M, et al. *J Pediatr* 1992;120:721-725.
27. Haack D, et al. *Pediatr Nephrol* 1999;13:653-656.
28. Sarna S, et al. *J Clin Endocrinol Metab* 1997;82:75-77.
29. Ferraris JR, Pasqualini T. *J Rheumatol* 1993;20(suppl 37):43-46.
30. Broyer M, et al. *Pediatr Nephrol* 1997;11:418-422.
31. Ortoft G, et al. *J Bone Miner Res* 1999;14:710-721.
32. Ward WE, Atkinson SA. *J Pediatr Gastroenterol Nutr* 1999;28:404-410.
33. Chrysis D, Underwood LE. *Endocrinology* 1999;140:5635-5641.
34. Horber FF, Haymond MW. *J Clin Invest* 1990;86:265-272.
35. Bennet WM, Haymond MW. *Clin Endocrinol* 1992;36:161-164.
36. Mauras N, Haymond MW. *Pediatr Nephrol* 1996;10:318-323.
37. Morris HG, et al. *J Clin Invest* 1968;47:427-435.
38. Davies UM, et al. *J Rheumatol* 1994;21:153-158.
39. Touati G, et al. *J Clin Endocrinol Metab* 1998;83:403-409.
40. Allen DB, Goldberg BD. *Pediatrics* 1992;89:416-421.
41. Allen DB, et al. *J Clin Endocrinol Metab* 1998;83:2824-2829.
42. Tönshoff B, et al. *Kidney Int* 1993;44:199-207.
43. Tönshoff B, Fine RN. In: Nissenson AR, Fine RN, Gentile DE, eds. *Clinical Dialysis.* Burr Ridge, Ill:McGraw-Hill. 1995:549-573.
44. Hokken-Koelega ACS, et al. *Lancet* 1994;343:1313-1317.
45. Fine RN, et al. *J Pediatr* 2000;136:376-382.
46. Broyer M, et al. *J Am Soc Nephrol* 1995;6:1075. Abstract.
47. Guest G, et al. *Pediatr Nephrol* 1998;12:437-446.

ERRATUM

Regarding question 3 of the post-test for *GGH* Volume 16, Number 1, neither statement is true. In familial male precocious puberty, aromatase inhibition slows growth rate and epiphyseal maturation.

Is Short Stature a Handicap? A Comparison of the Psychosocial Functioning of Referred and Non-Referred Children With Normal Short Stature and Children With Normal Stature

Heretofore, most reports that short stature conferred significant academic and social handicaps have utilized subjects who were referred for pediatric endocrinologic evaluation. The present authors evaluated the psychosocial status of 2 populations of children with normal short stature (NSS, or ISS; height below the National Center for Health Statistics [NCHS] 5th percentile not associated with illness, hormonal deficiency, or dysmorphic syndrome) in comparison to that of a third control group of children of average height. In 27 children with NSS referred for pediatric endocrinologic evaluation (group 1), mean height SDS was –2.7 (range, –4.5 to –1.3). In 34 nonreferred children with NSS (group 2) who were identified through a public school screening program, the mean height SDS was –1.7 (range, –3.2 to –1.3). For the 29 in the third group, the mean height SDS was 0.06 (range, –0.7 to +0.7). Tests of verbal and nonverbal intelligence (Kaufman Brief Intelligence Test [K-BIT]) and educational achievement (Kaufman Test of Educational Achievement [KTEA]) were administered. Family coherence and adaptability were assessed using the Family Adaptability and Cohesion Evaluation Scales (FACES II), as were the adaptive and problem behaviors (by the Behavior Assessment System for Children [BASC]).

No relationship was found between the height SDS and psychosocial functioning. The composite IQs of all 3 groups were similar (K-BIT). Composite and spelling achievement (KTEA) were similar in all groups, but the individuals in group 3 were significantly advantaged in mathematics and reading achievement over those in groups 1 and 2. As assessed by parents, the NSS subjects in group 1 had higher aggressivity, hyperactivity, conduct problems, and attention deficit scores, and lower social skill scores (BASC) than did nonreferred NSS or normal-statured students. Nonreferred ISS and normal height children (groups 2 and 3) had similar behavioral profiles. Teachers discerned no differences in adaptive or problem behaviors among the 3 groups. There were no intragroup differences in parental cohesion and adaptability (FACES II).

The investigators concluded that the results are consistent with the *hypothesis* that "discrepancies between earlier and more recent research on the psychosocial functioning of children with SS [short stature] may be explained, at least partly, by referral bias." These results also provide further evidence indicating that SS per se is not a handicapping condition.

Kranzler JH, et al. *J Pediatr* 2000;36:96-102.

Editor's comment: *This report confirms those of other investigators that NSS is not a "handicapping condition." The reason why average height children were more adept in mathematics and reading than NSS children in this study is not apparent. Could this reflect a gene or gene-associated phenomenon linking these 2 skills?*

*Voss and Saenger (*J Pediatr *2000;136:103-110) debate the usefulness of treatment of NSS with GH. Voss argues that treatment with GH is not justified on the basis of auxologic findings (short stature, slow growth rate) because "short-term growth data . . cannot reliably distinguish between normal and abnormal growth" and because "there is no correlation between successive annual height velocities, so that height velocity neither predicts the future nor reports the past." Voss continues that treatment does not appear justified on the basis of either psychological or learning disabilities. Voss discusses in his presentation the definition of "normality," and concludes that "differences are tolerable; deviance demands action."*

Saenger attempts to argue for treatment of NSS and to defer final judgment regarding treatment until more data have been accumulated on the auxologic and psychologic efficacy of therapy, a position that Voss effectively rebuts.

In a different article (Arch Dis Child *2000;82:10-15), Hall discusses the utility of growth screening in schools. He points out the many methodologic problems associated with measurements of height and suggests a height less than the 0.4 percentile (–2.67 SD) as the cutoff measurement below which it is reasonable to do further evaluation. He states that in a group of 400 children with height less than this percentile, as many as 30 children with isolated GH deficiency and 12 with Turner syndrome, and an additional group consisting of undiagnosed illnesses producing SS, will be identified.*

Allen W. Root, MD

2nd Editor's comment: *Readers are referred to* GGH *2000;16:1-5 to read the lead article, "Ethical Issues in Growth Hormone Therapy: Where Are We Now?" This article is based on a seminar workshop held at the University of Wisconsin in October 1999.*

Robert M. Blizzard, MD

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc. The information reflects the views of the editors and/or contributors and not necessarily those of the sponsor, grantor, or the publisher.

Published by:

Gardiner-Caldwell
SynerMed

405 Trimmer Road
PO Box 458
Califon, NJ 07830

Short Stature in Carriers of Recessive Mutation Causing Familial Isolated Growth Hormone Deficiency

The phenotype in patients with isolated GH deficiency (IGHD) type 1B is identical to patients with IGHD type 1A, which results from homozygous absence of the *GH-1* gene. Patients with type 1B have a loss of function mutation in the *GH-1* gene. Both 1A and 1B patients initially respond favorably to rhGH. However, they differ in that type 1A patients develop GH antibodies that then inhibit growth. Patients with type 1B do not develop GH antibodies and continue to respond.

These investigators report that family members who were heterozygous for the loss-of-function gene are frequently shorter than their homozygous normal relatives for the *GH-1* gene.

The authors studied an extended, interrelated Bedouin family with a G to C transversion at the 5th base in intron IV of *GH-1*, leading to loss of a splice site and utilization of a cryptic splice site in exon IV that resulted in loss of 73 bp and a nonfunctional 196 amino acid product. Among a sample of 50 first- and second-degree relatives of the 9 homozygous patients, 33 were found to be heterozygous for the *GH-1* mutation and 17 to be homozygous normals. The heterozygous subjects were significantly smaller than the normal individuals (−1.67 vs −0.40 SDS; $P>0.05$) without relation to sex or age. In 33% of the heterozygous group, heights were ≥2 SDS below the mean (Tables 1 and 2). Stimulated secretion of GH was normal in the heterozygous subjects tested.

The authors *hypothesized* that this mutation impaired transport of the product to the secretory granules and that there was subnormal spontaneous GH secretion. They concluded that the described mutation manifested itself as short stature in heterozygous subjects and suggested that this or similar mutations in *GH-1* in the heterozygous state might account for some of the phenotypic variability in population heights and for some of the patients with normal short stature encountered in the clinic.

Leiberman E, et al. *Am J Med Genet* 2000;90:188-192.

Table 1
Mean Standard Deviation Scores for Height in Heterozygotes and Normal Homozygotes According to Sex

	Heterozygotes Mean SDS (± SE)	Normal Homozygotes Mean SDS (± SE)	*P*
Males	−1.22 (± 0.28) *n* = 13	−0.10 (± 0.19) *n* = 11	NS
Females	−1.97 (± 0.29) *n* = 20	−0.95 (± 0.31) *n* = 6	NS
Total	−1.67 (± 0.21) *n* = 33	−0.40 (± 0.19) *n* = 17	<0.05

Reprinted with permission from Leiberman E, et al. *Am J Med Genet* 2000;90:188-192.

Editor's comment: *Limited clinical manifestations of rather severe autosomal recessive disorders are being recognized with increasing frequency. Some heterozygous relatives of patients with loss-of-function mutations of the GH receptor or of the GH-releasing hormone receptor may be inappropriately small, and occasional adult females who are heterozygous for loss-of-function mutations of* CYP21B *may manifest evidence of mild hyperandrogenism. The findings here may be of great significance in explaining some of the variation of stature that is seen in families.*

Allen W. Root, MD

Table 2
Mean Height (cm) and Mean Standard Deviation Scores (MSDS) for Height in Heterozygotes (H) Compare With Normal Homozygotes (N) According to Age Groups

	Adults		Adolescents		Children	
	H n = 19	N n = 6	H n = 5	N n = 5	H n = 9	N n = 6
Height (± SE)	158.6 (± 0.57)	169.3 (± 1.51)	143.4 (± 2.41)	157.4 (± 2.63)	105.2 (± 2.53)	126.5 (± 2.45)
MSDS (±SE)	−1.43 (± 0.23)	−0.21 (± 0.24)	−2.22 (± 0.67)	−0.90 (± 0.38)	−1.88 (± 0.51)	−0.18 (± 0.32)

Reprinted with permission from Leiberman E, et al. *Am J Med Genet* 2000;90:188-192.

Comparison of the Growth-Promoting Effects of Insulin-Like Growth Factor 1 and Growth Hormone in the Early Years of Life

The authors report that administration of rhGH to 4 young children with isolated GH deficiency (IGHD) due to deletion of *GH-1* increased linear growth rate to a greater extent than did administration of recombinant human insulin-like growth factor 1 (rhIGF-1) to 3 children with GH insensitivity (GHI). The mean birth length (Table) in the 4 children with IGHD was 46.5 cm (–3.5 SDS); in 5 GHI neonates, mean birth length was 46.8 cm (–3.3 SDS). During the first 2 years of life, length of untreated IGHD infants declined to –5.7 SDS, that of GHI infants from –3.5 to –6.5 SDS.

Treatment was initiated in all 4 IGHD patients and 3 of the 5 GHI patients between 1 and 4 years of age. With replacement rhGH treatment, the heights of IGHD children increased between 1.2 and 2.4 SDS over 3 years. In the 3 GHI children treated with rhIGF-1, height increased between 0.5 and 1.4 SDS over 3 years. The patient treated at the earliest age grew the least. By 2 years of age, head circumferences of all subjects were < –2.5 SDS; during administration of rhGH or rhIGF-1, head circumferences increased. The authors conclude that the linear growth response to rhGH is greater in young children with IGHD than the linear growth response to rhIGF-1 in subjects with GHI. This implies that both GH and IGF-1 are necessary for optimal linear growth during early childhood.

Laron Z, Klinger B. *Acta Paediatr* 2000;89:38-41.

Editor's comment: *Normal cartilage growth requires both GH and IGF-1. GH is thought to cause chondrocyte progenitor cells to differentiate and to increase local production of IGF-1; this growth factor then stimulates clonal expansion of proliferating and hypertrophic chondrocytes. Although rhIGF-1 markedly increases linear growth rates in prepubertal children with GHI, current observations suggest that both GH and IGF-1 are necessary for maximal growth in height, particularly in young children.*

Allen W. Root, MD

2nd Editor's comment: *The authors have presented data on only 7 patients receiving rhGH or, alternatively, rhIGF-1. There were 2 additional patients (GHI) who did not receive treatment. The study was worth doing, but possibly the results were overinterpreted, as the number of patients in each group was small (4 vs 3 vs 2 patients in each group), the doses of rhGH and IGF-1 were not proven to be biochemically equivalent, and patient ages at treatment were not paired for the group. The value of the article to me is the confirmation that birth weights and lengths are pathologically small in all patients reported, as were head sizes; that growth rates increase significantly in IGHD patients receiving rhGH and in GHI patients receiving rhIGF-1; and that individual variation of response, as exemplified by the observation that the poorest response to rhIGF-1 occurred in the youngest patient to receive the hormone at the largest dose, makes comparison of response between groups of such limited number difficult.*

As an incidental comment, the head circumferences of IGHD and GHI patients are significantly small. While head circumference does increase with treatment, there are no data to my knowledge suggesting that the increase in head size (presumably brain size) affects intellectual capability. Also of great interest to me is that the heights of the parents, who undoubtedly are heterozygotes for IGHD or GHI, are in the negative SDS range (Table). The question of heterozygosity for certain genes affecting stature is addressed in the previous abstract.

Robert M. Blizzard, MD

Table

Pertinent Clinical Data at Referral of 4 Patients With Congenital Isolated Growth Hormone Deficiency (IGHD) and 5 Patients With Laron Syndrome (LS)

					Birth Length		Parents' Height (SDS)	
No.	Sex	Diagnosis	CA (y)	BA (y)	(cm)	SDS†	Mother	Father
1	F	IGHD	3.9	2.5	46	–3.5	–1.45	–1.13
2	F	IGHD	3.2	0.7	45	–4.0	–1.61	–1.53
3*	M	IGHD	1.2		46	–4.0	–3.45	–2.66
4* Siblings	M	IGHD	0.9	0.2	49	–2.5	–3.45	–2.66
5	F	LS	3.6	2.5	48	–2.5	–0.30	–0.59
6	M	LS	0.6	0.2	45	–4.5	–0.65	–1.16
7 Siblings	M	LS	3.4	1.5	45	–4.5	–0.65	–1.16
8	F	LS	2.8	1.0	49	–2.0	–0.70	–1.61
9 Siblings	M	LS	1.0	0.5	47	–3.0	–0.70	–1.61

*Mother also is IGHD. †According to Tanner et al. CA, chronologic age; BA, bone (skeletal) age.

Fetal Alcohol Syndrome and Brain Receptors

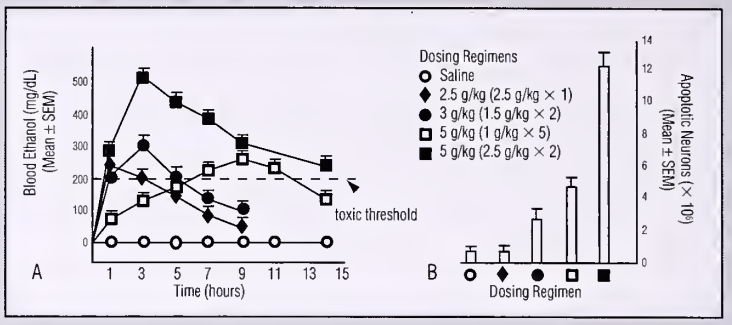
Intrauterine exposure of the human fetus to ethanol damages the developing brain, producing fetal alcohol syndrome (FAS) or fetal alcohol effects (FAEs), depending on severity. The primary manifestations are neurobehavioral disturbances, ranging from hyperactivity and learning disabilities to depression and psychosis. Patients severely affected also exhibit characteristic facies and growth deficiency. It long has been suspected that sensitivity to ethanol correlates with the time when synapses form, which is greatest during the last trimester of gestation for humans. A study headed by John Olney and colleagues provides an explanation for this correlation and identifies a probable mechanism that contributes to FAS/FAEs.

This work was done in rats, in whom the period of synaptogenesis occurs postnatally. Ethanol exposure of 1-week-old rats leads to a generalized loss of brain mass and a specific loss of cerebellar and hippocampal neurons. The authors had previously observed that transient blockade of *N*-methyl-D-aspartate (NMDA) glutamate receptors during the period of synaptogenesis causes widespread apoptosis of neurons in the infantile rat brain. Since ethanol is a known NMDA antagonist, Olney and colleagues explored the possibility that apoptosis is the mechanism by which ethanol causes neuronal loss (Figure).

Examination of brains 1 day after exposure to a control injection of saline revealed a low level of apoptosis consistent with the normal process by which biologically redundant neurons are deleted during brain development. However, after ethanol exposure, apoptosis was extensive. When quantitated by neuronal density, degenerating neurons comprised 0.13% to 1.55% of the total neurons in controls compared with 5% to 30% in ethanol-exposed brains. The extent of apoptotic degeneration varied by region. Dosing experiments revealed a threshold for apoptotic changes; blood ethanol concentration had to remain above 200 mg/dL for 4 hours to induce apoptosis. Exposures beyond this threshold led to progressively more severe apoptotic degeneration. They also found a time window from near the end of gestation to 2 weeks of age during which neurons in the forebrain showed transient sensitivity to ethanol. The period of vulnerability varied slightly among different populations of neurons, but coincided with the time when synapses were being formed.

The authors also screened for other drugs that could induce apoptosis of neurons. They found that drugs that block the NMDA receptor for glutamate, which is an excitatory neurotransmitter, or those that activate receptors for the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), trigger apoptosis of neurons during the time of synaptogenesis. The most relevant drugs in this category were benzodiazepines and barbiturates. The authors caution that even though the window of greatest sensitivity in humans to ethanol and other drugs that block NMDA glutamate receptors or activate GABA receptors is the last trimester of pregnancy, synapses continue to form for several years after birth. They point out that prolonged use of these drugs as anticonvulsants in infants could pose a risk to the developing brain.

Figure



Ethanol was administered to P7 rats by several dosing regimens. The total dose ranged from 0 to 5 g/kg SC and was administered either in a single injection or in multiple injections spaced 2 hours apart. (A) Blood ethanol curves associated with each of several dosing regimens. (B) Severity of apoptotic neurodegeneration associated with each dose–blood ethanol curve.

Reprinted with permission from Ikonomidou C, et al. *Science* 2000;287:1056-1060.

Ikonomidou C, et al. *Science* 2000;287:1056-1060.

Barinaga M. *Science* 2000;287:947-948. News.

Editor's comment: *This paper provides new insight into the mechanism by which ethanol harms the developing fetus. It offers potential explanations for why binge drinking, with its sustained high levels of ethanol, as well as drinking in late pregnancy, after organogenesis is largely completed, can have such severe consequences on the developing brain. A potential danger of misinterpretation in this article is to conclude that drinking small amounts of ethanol in the early and middle stages of pregnancy is not harmful. This is unwarranted given the many aspects of the mechanism uncovered here that remain poorly understood, and the substantial differences in nervous system development between rats and humans. Knowing how ethanol disturbs neuronal development provides the first step to devising ways to prevent or minimize its harmful effects on the unborn.*

William A. Horton, MD

A Novel Subtype of Type 1 Diabetes Mellitus Characterized by a Rapid Onset and an Absence of Diabetes-Related Antibodies

Type 1 diabetes mellitus is classified as type 1A (autoimmune) or idiopathic (type 1B). The second is less frequent, and little is known concerning the entity. This article deals with type 1B, which in turn may be 2 different diseases, as elucidated by these investigators. Imagawa et al classified 56 consecutive Japanese adults with type 1 diabetes according to the presence or absence of glutamic acid decarboxylase (GAD) antibodies as a marker of autoimmunity. Thirty-six of 56 patients had GAD antibodies, indicative of type 1A diabetes; 20 patients did not. On the basis of elevated versus low glycosylated hemoglobin values, the 20

patients with type 1B were divided into 2 groups: 11 with low values and 9 with high values (Figure). Among the 56 consecutive Japanese patients, 11 were identified with a subtype of diabetes differing from autoimmune diabetes (type 1A) in 3 respects. No autoimmune features were detected, and no diabetes-related serum antibodies such as islet cell, GAD, or insulin antibodies—as occur in type 1A—were detected. Also, neither insulinitis nor hyperexpression of MHC class I molecules was found in the islets when pancreatic biopsies were performed in 3 patients.

These patients differed from the usual patients with type 1B with respect to the low glycosylated hemoglobin values and the rate of clinical onset, which was rapid. Diabetic ketoacidosis occurred less than a week after the onset of hyperglycemic symptoms, while patients in the other 2 groups (type 1A and usual type 1B) had symptoms several weeks before ketoacidosis appeared. The normal glycosylated hemoglobin values in the 11 type 1B subgroup patients probably reflected the short duration of hyperglycemia. Insulin secretory capacity estimated on the basis of urinary C-peptide excretion was significantly lower in these than in the other patients, and the metabolic derangement at the onset was severe. These 11 patients also differed in a third way. The serum pancreatic enzyme concentrations were markedly elevated, which was in accord with lymphocytic infiltration of the *exocrine* pancreas seen in the biopsy specimens obtained. Patients in the other 2 groups had normal serum pancreatic enzyme concentrations and apparent insulinitis (as determined by the limited number of biopsies performed), but did not have the lymphocytic infiltrates in the *exocrine* pancreas found in the 3 patients in this subgroup of 11. The edema, necrosis, hemorrhage, suppuration, cyst formation, and fibrosis that characterize classic acute or chronic pancreatitis were not present.

On the basis of these findings, the investigators believe that diabetes characterized by the absence of GAD antibodies and the presence of low glycosylated hemoglobin values should be classified as nonautoimmune, fulminant type 1 diabetes, a subtype of idiopathic type 1B diabetes.

The investigators state that the precise mechanism of beta-cell destruction in this subtype of diabetes is unknown. However, they suggest a viral cause because of the abrupt onset of diabetes, the presence of lymphocytic infiltrates in the exocrine pancreas, and the affinity of several viruses for exocrine pancreatic tissue. Further studies with younger patients and other ethnic groups may provide a better understanding of this subtype of type 1B diabetes.

Imagawa A, et al. *N Engl J Med* 2000;342:301-307.

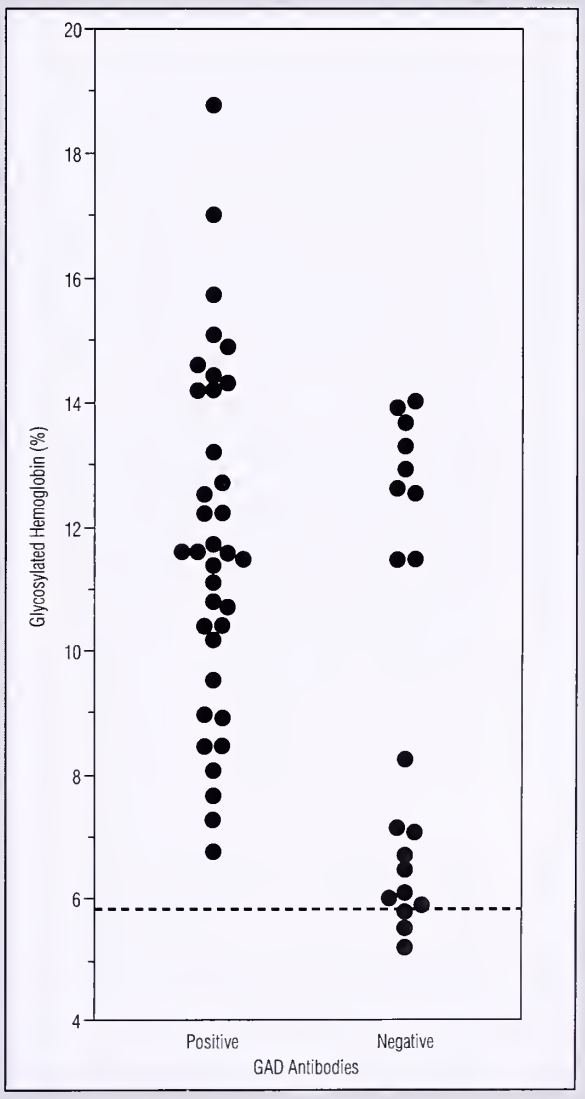
Editor's comment: *This very interesting article clearly defined a new subgroup of type 1 diabetes mellitus in adults of Japanese origin. It remains to be determined whether this subtype is common in whites or blacks. In the article by Imagawa et al, 64% of patients with adult-onset diabetes had type 1A (36 of 56 patients), and approximately half of the remaining 20 patients had different types of what is now called type 1B diabetes. The precise mechanism of beta-cell destruction in the 2 subtypes of type 1B remains undetermined, and very likely will be different for these 2 subgroups. With respect to a viral infection in preliminary studies by Imagawa et al, no viral antibodies were found, but these were preliminary studies. Fulminant type 1 diabetes is very rare in white, Anglo-Saxon individuals.*

The reader interested in diabetes as a clinical entity, especially its origin and its genetics, is encouraged to read this article in its entirety. The accompanying editorial by Dr. Hake Lernmark in the New England Journal of Medicine *offers additional insightful comments.*

Fima Lifshitz, MD

Figure

Glycosylated Hemoglobin Values at the Time of the Diagnosis of Diabetes in 56 Patients, According to Whether the Test for Glutamic Acid Decarboxylase (GAD) Antibodies Was Positive or Negative



The values for glycosylated hemoglobin in the patients with positive antibody tests are scattered, whereas the values in the patients with negative antibody tests are clearly divided into 2 groups: those below 8.5% and those above 11.5%. The broken line indicates the upper limit of the normal range for glycosylated hemoglobin.

Reprinted with permission from Imagawa A, et al. *N Engl J Med* 2000;342:301-307.

Transient Neonatal Diabetes Mellitus

Neonatal diabetes mellitus (NDM) occurs in both a transient (TNDM) and permanent (PNDM) form. Some cases of TNDM occur because of *paternal* uniparental isodisomy (UPD) of chromosome 6. Such UPD has not been demonstrated in PNDM.

Hermann et al studied 6 patients with NDM: 3 with TNDM and 3 with PNDM. Microsatellite markers and human leukocyte antigen alleles were examined using polymerase chain reaction and DNA fragment electrophoresis. Humoral markers of islet cell autoantibodies also were studied. Of the 6 patients with NDM, 1 of the 3 with TNDM and macroglossia carried UPD of chromosome 6. No maternal chromosome 6 sequences were present.

In the 3 patients with PNDM and the other 2 patients with TNDM, no evidence for UPD could be found. None of the 6 had the high-risk type 1 diabetes human leukocyte antigen alleles. Only 1 patient had islet-specific autoantibodies, but did not have glutamic acid decarboxylase antibodies, which are the antibodies most indicative of autoimmune diabetes mellitus. The conclusion by Hermann et al was that patients with transient and permanent forms of NDM have different genetic backgrounds and represent different disease entities. TNDM is often associated with UPD of chromosome 6, suggesting that an imprinted gene on chromosome 6 is responsible for this phenotype. It seems that 2 copies of the paternal allele are necessary for the development of TNDM in the cases with paternal UPD; therefore, it is likely that overexpression of a putative gene located on chromosome 6 alters pancreatic beta-cell maturation and insulin secretion.

The article by Christian et al reports 2 cases of NDM: 1 with PNDM and 1 with TNDM. The latter had macroglossia, which has been reported in some of the 7 previously published cases. These authors suggest macroglossia in the presence of NDM is an unequivocal indicator to search for UDP of chromosome 6.

Hermann R, et al. *Pediatrics* 2000;105(1):49-52.

Christian SL, et al. *J Pediatr* 1999;134(1):42-46.

Editor's comment: *NDM is a rare disorder, with an estimated incidence of 1 in 400,000 live births. The 3 patients with PNDM had normal biparental inheritance of chromosome 6, and 1 of the 3 with TNDM had demonstrable UPD of chromosome 6. Cases of TNDM without UPD of chromosome 6 may have mutations of a parental gene on chromosome 6, or some other explanation may exist. Genes on chromosome 6 appear to be involved with the development of beta-cell differentiation and/or maturation of the pancreas. Studies for UDP of chromosome 6 should be performed in all cases of NDM.*

Fima Lifshitz, MD

2nd Editor's comment: *Numerous reports now exist distinguishing NDM from other forms of diabetes. Because of the good prognosis, it is suggested that it is worth screening for paternal UDP of chromosome 6 in all cases of NDM. I certainly am in accord with this recommendation.*

The mechanism by which insulin is controlled is obviously very complex. Insulin maps to chromosome 11. In the yolk sac, only the paternal insulin gene is expressed in mice. During embryonic development, there is usually biparental expression. However, something on chromosome 6 has to do with control of insulin expression at the time of birth, since UPD can lead to lack of expression from both insulin genes (ie, both the maternal and paternal genes on chromosome 11). Between 6 months and 3 years of age, a different mechanism must control insulin expression, since children outgrow their transient neonatal lack of insulin. This is what happens in patients with TNDM. Normally, in adults there is biparental expression of insulin in the pancreas.

It is of interest that the case with UDP reported by Hermann and colleagues also had macroglossia, which of course occurs in Beckwith-Wiedemann syndrome. Interestingly, in that syndrome there is overgrowth and hyperinsulinemia associated with the macroglossia and paternal UPD for chromosome 11. In the patients with NDM, birth weights are low or low normal.

Judith G. Hall, OC, MD

Incidence of Diabetes Mellitus and Impaired Glucose Tolerance in Children and Adolescents Receiving Growth Hormone Treatment

Cuttfield and colleagues investigated 85 cases of diabetes mellitus, abnormal glucose tolerance, and hyperglycemia reported to the Pharmacia and Upjohn International Growth Study (KIGS) database between 1987 and 1997. The KIGS database is an international pharmacologic survey of the safety and efficacy of GH therapy in children and adolescents. The database includes more than 23,000 children. The information regarding date of diagnosis, presenting symptoms, family history, measurements of antibodies, oral glucose tolerance testing, and risk factors for diabetes was recorded. Data were categorized using the American Diabetes Association (ADA) Expert Committee recommendations for the definition of diabetes. The observed incidence of type 1 diabetes was compared with information available in 12 of the different countries from which the GH data were extracted. The incidence of type 2 diabetes was matched by age to data from recently reported studies of type 2 diabetes in children from Cincinnati, Ohio, and Japan.

Using the ADA Expert Committee criteria, 42 of the 85 cases reported with abnormal glucose tolerance had to be excluded. Of the 43 remaining cases, 11 were diagnosed with type 1 diabetes, 18 with type 2 diabetes, and 14 with glucose intolerance. Three of the type 1 patients had ketosis, 3 had islet cell antibodies, and 3 had low secretion of C-peptide. In the 18 children who developed type 2 diabetes, 7 had at least 1 risk factor for diabetes. All had persistent diabetes after GH therapy was stopped. The incidence and age at diagnosis of children treated with GH were not different from expected values. However, the

incidence of type 2 diabetes was significantly higher in the adolescents aged 10 to 19 years: 46.3 per 100,000 years of GH treatment, versus 7.2 per 100,000 years of GH treatment in 10- to 19-year-old adolescents from Cincinnati. The children aged 6 to 14 years with type 2 diabetes had an incidence of 27 per 100,000 years of GH treatment, which was greater than that found among Japanese children in the same age range (4.6 per 100,000 years of GH treatment).

The authors express concern that the incidence of type 2 diabetes was *6-fold higher* in children treated with GH compared with published controls. They point out that the treatment database used for this study lacks a prospectively acquired control population. They also point out that their data cannot be compared with that from the National Cooperative Growth Study (NCGS) of children treated with GH in the United States, since the published reports from that study did not distinguish between different types of diabetes. They warn, however, that since they used the very strict diagnostic criteria of the ADA Expert Committee, they excluded the diagnosis in almost half of their original 85 subjects.

Cuttfield W, et al. *Lancet* 2000;355:610-613.

Editor's comment: *Clearly, this report from the KIGS database presents information that differs from that of the NCGS database* (J Clin Endocrinol Metab 1996;81:1704-1710). *The reasons for these differences may be attributed to the larger number of countries from which the data are being collected in the current report and the use of more recent ADA Expert Committee diagnostic criteria for types 1 and 2 diabetes and impaired glucose tolerance. It is important to point out, as the authors did, that patients with some of the underlying diagnoses, such as Turner syndrome, intrauterine growth retardation, and Prader-Willi syndrome, are already at high risk of developing type 2 diabetes. The authors do not separate out these individuals in their results. Since there is a well-recognized increasing incidence of type 2 diabetes among children and adolescents, utilizing retrospective and country-specific controls may not be appropriate. Regardless, a 6-fold higher increased incidence is highly significant.*

An accompanying editorial by William Jeffcoat, Nottingham, utilizes the data to caution physicians about treating adults with GH. By virtue of age and other risk factors, these adults may already be at significant risk for type 2 diabetes.

These data will have worldwide significance. It is important that pediatric endocrinologists become familiar with these results and their implications. This will lead to better and longer observation of children treated with GH. These data cannot be dismissed and, clearly, the tacit implication is that children undergoing therapy with rhGH should be screened periodically for glucose intolerance both during therapy and after therapy if therapy is stopped when patients reach adulthood.

William L. Clarke, MD

Birth Weight and the Insulin Resistance Syndrome: Association of Low Birth Weight With Truncal Obesity and Raised Plasminogen Activator Inhibitor-1 but Not With Abdominal Obesity or Plasma Lipid Disturbances

The insulin resistance syndrome was defined as the combination of hypertension, insulin resistance, and dyslipidemia. Clusters of physiologic factors, including hypertension, impaired glucose tolerance, insulin resistance, lipid disturbances, and impaired fibrinolytic activity, were studied intermittently from birth to 70 years of age in males. These factors are related to birth weight among a large cohort of adult men studied in the Uppsala, Sweden Longitudinal Study of Adult Men. This study included all men born between 1920 and 1924 and still living in Uppsala. These men were studied between 1970 and 1973, at an age of approximately 50 years, and again in 1991, at an age of approximately 70 years. The investigators were able to trace birth records of more than 1,300 of the participants in the original study (n=24,841), and selected cutoffs for birth weight for their studies (<3.25 to 3.75, 3.75 to 4.25, > 4.25 kg). The phenomena investigated at age 50 (n = 1,268) included height, weight, skinfold measurements, blood pressure, intravenous glucose tolerance tests, and blood lipids. At age 70 (n = 734), in addition to an oral glucose tolerance test, a euglycemic hyperinsulinemic clamp study was performed and plasminogen activator inhibitor-1 (PAI-1) activity was assessed. The latter is a marker of impaired fibrinolytic activity. Information on smoking and socioeconomic factors was recorded.

Of the men studied at age 50, type 2 diabetes occurred in 2%, and 26% were hypertensive. At age 70, 14% had type 2 diabetes, and 67% were hypertensive. At age 50, triceps skinfold thickness was positively associated with birth weight. Inverse relationships between birth weight, fasting insulin, and insulin resistance previously have been published, as have positive associations with bone mineral density at age 50 and insulin sensitivity at age 70. When adjusted for body mass index, birth weight was inversely related to waist-hip ratio, PAI-1 activity, and insulin and proinsulin concentrations. Serum triglyceride concentrations and high-density lipoprotein cholesterol levels were not significantly associated with birth weight. Socioeconomic factors and smoking history did not change the relationship of birth weight with the insulin resistance factor.

The authors point out that they have demonstrated strong associations between reduced size at birth, adult hypertension, insulin resistance, glucose intolerance, cardiovascular mortality, and high PAI-1 activity. A strong relationship has been demonstrated between birth weight and insulin resistance syndrome in British men, aged 64 years, and US men at age 31.5 years. The participants in those studies were more obese than those in the current study, and the authors state that this could mediate the stronger effect of birth weight on the insulin resistance syndrome in those groups. Finally, the authors question why low birth weight predicts only some components of the insulin resistance syndrome and not others, and what the physiologic pathways linking these disturbances might be.

Byberg L, et al. *Diabetologia* 2000;43:54-60.

Editor's comment: *Pediatricians, generalists, and internists need to be aware of the deleterious effects of low birth weight on the subsequent morbidity and mortality of adults. These carefully collected and analyzed epidemiologic data of adult men are exceedingly important. Although socioeconomic factors did not influence the incidence of the insulin resistance syndrome, it remains unclear whether personal psychological factors such as the desire to feed and/or overfeed a small baby and/or the need to restrict caloric intake in the large infant might have a bearing on subsequent outcomes.*

The findings that smoking and socioeconomic status did not influence the results are of particular importance. As the insulin resistance syndrome and type 2 diabetes, in particular, have become more prevalent both in children and adults, it is important that significant effort be placed into determining factors that contribute to the onset and persistence of these adversities. A better understanding of the relationships between the variables presented in this article is needed, and perhaps the nutritional principles taught to pediatric residents need to be carefully reviewed if a significant impact is going to be made in the reduction of the near-epidemic disorder of insulin resistance in children.

William L. Clarke, MD

Mosaicism Is a Likely Explanation for the Variability Observed in Androgen Insensitivity Syndrome

Holterhus et al report on 5 patients with somatic mosaicism for abnormalities of the androgen receptor. In all 5 patients, there was a lack of family history; and in all 5 clitoromegaly or micropenis with scrotalization of the labia was present. Each of the 5 patients had a different mutation that had arisen during postzygotic development. It appears that somatic de novo mutations of the androgen receptor occur at a particularly high rate. Thus, somatic mosaicism should be considered when there is more masculinization than expected from a particular mutation.

It is desirable to study both blood leukocytes and tissue fibroblasts to determine whether an individual is mosaic. Individuals with androgen insensitivity mosaicism may need to undergo early gonadectomy in order to avoid further masculinization. Variable expression of wild-type gene products, based on somatic mosaicism, is probably the mechanism for much of the variability that is seen in androgen insensitivity syndrome.

Holterhus P-M, et al. *Pediatr Res* 1999;46:684-690.

Editor's comment: *The more we study, the more we learn. Somatic mosaicism appears to be quite common in a number of disorders, but seems to be variable depending on the particular gene. It is important to be aware that the androgen receptor gene seems to be particularly mutable during the course of development and, thus, we can explain the variability seen as related to a specific mutation. Geneticists like to think that there can be genotype-phenotype correlations that are straightforward, but somatic mosaicism leads to confounding situations. Keep an eye out for unexpected variations and consider somatic mosaicism as a possible explanation.*

Judith G. Hall, OC, MD

Perceptions of the Outcome of Orthopedic Surgery in Patients With Chondrodysplasias

Hunter has taken the time to carefully interview 197 individuals with disproportionate short stature. Seventy-four of the 197 had undergone a total of 221 surgical procedures. In general, individuals felt they had improved outcomes. However, the attitude very much depended on the particular disorder. The percentage of individuals undergoing surgery ranged from 8.3% for hypochondroplasia to 87.5% for diastrophic dysplasia. The worst outcomes were for foramen magnum-cervical surgery and the best for thoracolumbar procedures to release nerve compression.

Gross points out in his editorial that leg lengthening has been revolutionized by the Ilizarov technique. The complication rate has dropped dramatically as experience has increased, decreasing to only 7% for patients with leg lengthening related to leg length discrepancy or short stature.

Hunter AGW. *Clin Genet* 1999;56:434-440.
Gross R. *Lancet* 1999;354:1574-1575. Editorial.

Editor's comment: *Quality-of-life issues have become very important in healthcare outcomes analysis. Clearly, many patients reported by Hunter indicated that they experienced major improvements from orthopedic procedures. However, the perceptions related to specific disorders. The outcomes and natural history also must be related to specific disorders.*

Most of the leg lengthening experience in disproportionate short stature is related to hypochondroplasia and achondroplasia (ACH). The procedure can add an extra 4 to 6 inches, which can make an enormous difference in the daily life of individuals whose height is approximately 4 ft. It is terribly important that data continue to be accumulated and combined since each type of disproportionate short stature is relatively rare. Collaborative studies on an international basis are really needed.

Judith G. Hall, OC, MD

2nd Editor's comment: *Physicians dealing with short stature need to be aware of these 2 articles and the lead article by Dr. Deborah F. Stanitski, "Limb Lengthening in the Skeletal Dysplasias and Short Stature Conditions: State of the Art in 1997," that appeared in* GGH *(1997;13[2]:17-22). You are invited to read again the excellent presentation by Dr. Stanitski.*

The article by Hunter is a general article, reporting levels of patient satisfaction for procedures performed for 2 different types of chondrodysplasias. The information will be of use to you in helping patients who are contemplating surgery, whether of the spine or extremities. However, it will not tell you the information you need to advise the patient.

The commentary by Gross in Lancet *may be more helpful to*

*pediatric endocrinologists and geneticists than the article by Hunter, as it deals with limb lengthening. He presents the historical aspects and then presents a concise summary of Ilizarov's contributions in the 1980s. The Verona surgeons have now described their results in 230 tibial lengthenings by monolateral fixation performed between 1990 and 1995; 58 were in ACH patients. Using these data, he points out that 40 days in a fixator was required for 1 cm of lengthening, or 200 days for 5 cm. The complication rate is now much less than previously, and Aldegheri reports (*J Bone Joint Surg [Am] *1999;81:624-634) that only 7% of patients had complications undergoing lengthening with his latest modification in technique. Gross points out that physical and mental scores for adults with ACH do not differ from the general population until about age 40, when back pain, weakness, and arthritis become disabling. Whether the effect of leg lengthening will speed up or delay this process in ACH, or adversely affect hip anatomy and function, remains unknown. Gross also comments:*

> *If tibial lengthening is successful in a patient with ACH, treatment remains incomplete until the femur and humerus have also been successfully lengthened. The financial and physical costs are substantial and there simply is no follow-up information to justify routine lengthening of several long bones. Thus, despite the gratifying improvements, the results of these procedures still need long-term evaluation and review.*

Robert M. Blizzard, MD

Russell-Silver Syndrome Begins to Be Unraveled

Russell-Silver syndrome is a very common diagnosis associated with intrauterine growth retardation.[1] However, it has become clear that it is a heterogenous disorder. Approximately 10% of cases have been associated with maternal uniparental disomy for chromosome 7. This observation suggests there are genes on chromosome 7 that are imprinted. *MEST* (also known as *PEG1*) is an imprinted gene expressed only from the paternal allele, which maps to human chromosome 7q.[2] Thus, patients who have maternal uniparental disomy lack paternal activation of the gene.

Lefebvre and coworkers disrupted the murine homologue, *Mest,* by gene targeting in embryonic stem (ES) cells.[2] The targeted gene was imprinted and reversibly silenced by passage through the female germ line. Paternal transmission activated the allele and caused embryonic growth retardation. Interestingly, *Mest*-deficient females showed abnormal maternal behavior, including impaired placentophagia. Thus, in mice, both growth and behavior are affected.

Interestingly, imprinting of *PEG1/MEST* is lost in lymphocytes and transformed lymphoblastoid cell lines. This is not entirely surprising since genomic imprinting is usually regulated in a tissue-specific way. In addition, imprinting may be controlled in a promoter-specific way such that promoters allow expression from a particular parental allele.[3] Imprinting can be governed in an isoform-specific way such that a single transcription unit will encode for different proteins via alternative splicing. Kosaki et al[4] demonstrate that there are different isoforms in lymphoblastoid tissue where isoform 1 is expressed only from the paternal allele while there is biallelic expression of isoform 2. Interestingly, there may be differences in mouse and human expression of isoforms, again in a tissue-specific way.

In addition to the paternally expressed *PEG1/MEST* gene in the 7q32 region, there also is a *g2-COP* gene[5] with biallelic expression in fetal brain and liver and in adult peripheral blood, and monoallelically paternal expression in other fetal tissues, including the skeleton, muscle, skin, kidney, adrenal glands, placenta, intestine, lung, chorionic plate, and amnion. Absence of paternal *g2-COP* transmission during embryonic development may contribute to the Russell-Silver phenotype. It may well be that other imprinted genes are present in this chromosome region. However, the expression is clearly under tight control in terms of tissue specificity and time of expression in development.

Duplication of 7p11.2-p13 also has been described as resulting in the Russell-Silver phenotype. The report by Monk et al[1] describes a chromosomal duplication within the region where gene *GRB10* (growth factor receptor-binding protein 10) has been identified. This suggests that Russell-Silver syndrome could result from overexpression of a maternally expressed imprinted gene as well as absence of a paternally expressed gene.

1. Monk D, et al. *Am J Hum Genet* 2000;66:36-46.

2. Lefebvre L, et al. *Mest. Nat Genet* 1998;20:163-169.

3. Lefebvre L, et al. *Peg1. Hum Mol Genet* 1997;6:1907-1915.

4. Kosaki K, et al. *Am J Hum Genet* 2000;66:309-312.

5. Blagitko N, et al. *Hum Mol Genet* 1999;8:2387-2396.

Editor's comment: *Imprinted genes seem to lie in regions where there are both maternally and paternally imprinted genes. As the Human Genome Project proceeds, we should be able to identify all genes in a given region. It does seem that many regions are very complex and that each may be under different types of control. Clearly, chromosome 7 has something very important to do with growth and behavior since either deficiency of paternal expression or the duplication of maternal genetic material can lead to important changes in growth and behavior. Other forms of growth retardation very possibly are attributable to the imprinting phenomenon.*

Judith G. Hall, OC, MD

Please Send Correspondence to:

Robert M. Blizzard, MD
University of Virginia, The Blake Center
1224 West Main Street, 7th Floor, Suite 701
Charlottesville, VA 22903

Post-Program Self-Assessment/CME Verification

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Follow the instructions listed there to receive CME Category 1 credit. Please note that each question may have more than one correct answer.

1. rhGH at a dose of 0.1 mg/kg/d in healthy adult volunteers _____ the protein catabolic side effects of prednisone.
   a. did not affect
   b. enhanced
   c. diminished
   d. prevented

2. Recent studies suggest that rhGH can produce positive effects on growth in growth-retarded children receiving glucocorticoids in which of the following:
   a. rheumatoid arthritis
   b. prepubertal children with renal allografts
   c. prepubertal adolescents with renal allografts

3. Independent factors that predicted the response to rhGH therapy in patients with chronic renal failure receiving glucocorticoids are:
   a. the degree of growth velocity prior to rhGH treatment (negative relationship)
   b. the degree of growth velocity prior to rhGH treatment (positive relationship)
   c. low glomerular filtration rate (positive relationship)
   d. the degree of insulin resistance prior to rhGH treatment (negative relationship)
   e. other

4. The authors state that _____ is known about the gene activity that eventually leads to growth retardation with glucocorticoid treatment.
   a. much
   b. a moderate amount
   c. little

5. Glucocorticoids _____ sulfation of cartilage matrix in the growth plate.
   a. paradoxically stimulate
   b. inhibit
   c. have no effect on

Answer Key: 1. d 2. a, b, c 3. a, d, e 4. c 5. b

CME Accreditation Statement

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

UNIVERSITY OF VIRGINIA HEALTH SCIENCES CENTER
SCHOOL OF MEDICINE

The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Disclosure: As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Mehls, Tönshoff, Lifshitz, Clark, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Inc.'s National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

GROWTH, Genetics, & Hormones is published under an unrestricted educational grant from Genentech, Inc.

Robert M. Blizzard, MD
c/o Gardiner-Caldwell SynerMed
405 Trimmer Road
PO Box 458
Califon, NJ 07830

GROWTH
Genetics & Hormones
Vol. 16 No. 3 November 2000

Maturity-Onset Diabetes of the Young (MODY): The Past, Present, and Future

Doris A. Stoffers, MD, PhD
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

INTRODUCTION

The entity maturity-onset diabetes of the young (MODY) was first recognized 4 decades ago among young diabetic patients who had a distinct clinical phenotype and inheritance pattern of diabetes mellitus (DM).[1] Patients with this entity characteristically differed from the usual young patient with type 1 DM in having a slow rather than an abrupt onset of DM, by not requiring insulin, by not having the severe symptoms of type 1 DM, and by having an autosomal dominant inheritance pattern. These observations led to the development of criteria to establish the diagnosis of MODY and distinguish it from type 1 DM. The strict application of these criteria (Table 1) resulted in the characterization of large multigenerational pedigrees that has greatly facilitated genetic studies during the last 5 years. Dramatic progress as a result of positional cloning efforts and candidate gene approaches led to the identification of several of the defects that underlie this early-onset form of type 2 DM. At least 5 forms of MODY have been elucidated to date. Reexamination of populations with common late-onset type 2 DM indicates that mutations in MODY genes may be associated with type 2 DM in general.

Since MODY is almost universally associated with an insulin secretory defect, these observations challenge the widely held belief that insulin resistance determines the expression of the diabetic phenotype. Rather, most of the patients with MODY and a significant portion of those with adult-onset type 2 DM have a genetically programmed impairment in the capacity of the pancreatic β cells to accommodate peripheral tissue insulin requirements.

Table 1
MODY Diagnostic Criteria

- Onset <25 years
- Autosomal dominant inheritance (3 generations)
- Not insulin requiring for ≥5 years after initial presentation

Reprinted with permission from Stoffers DA. In: *Current Review of Diabetes*, Simeon Taylor. Philadelphia, Pa: Current Medicine 1999:11-21.

The purpose of this review is to present MODY as a paradigm for type 2 DM, discuss the role of genetic screening in diagnosing and enhancing treatment of MODY, relate the identification and function of the 5 currently identified MODY genes, and discuss what we may learn about MODY from identification of other MODY genes and their mutations.

In This Issue

This educational activity is supported by unrestricted educational grants from Genentech, Inc. and Schwarz Pharma AG.

MODY As a Paradigm for Type 2 DM

The advances in our understanding of MODY are relevant to our understanding of the genetics and pathophysiology of type 2 DM in general. This is exemplified by the identification of mutations in MODY genes in subjects with late-onset type 2 DM.[2-4] Furthermore, there is often variation within MODY pedigrees with regard to the age of onset such that multiple affected members in some MODY pedigrees may have onset of disease after age 40. While the later age at diagnosis sometimes reflects a delay in ascertainment because of a mild phenotype, it also suggests that other factors, both genetic and environmental, may modify the expression of diabetes due to specific mutations in MODY gene loci. This leads to the consideration that mild mutations or polymorphisms in MODY genes may result in only a slight impairment of protein function and, therefore, may contribute to the expression of diabetes in a polygenic context. A common polymorphism at codon 98 of the *HNF-1α* gene *(MODY3),* which is not linked to DM in a Mendelian fashion, is nevertheless associated with reduced serum C-peptide and a reduced insulin response to glucose challenge. The prevalent yet incompletely penetrant D76N mutation in *IPF-1 (MODY4)* is associated with marked impairment in insulin secretion even in normal glucose-tolerant carriers of the mutation. Digenic inheritance in a family with late-onset type 2 DM has been documented in which the severity of the diabetic phenotype appears to relate to the cosegregation of 2 distinct mutations in 2 different pancreatic transcription factor genes, *IPF-1 (MODY4)* and *IB1,* a transcriptional regulator of *GLUT2* gene expression. Mutations that impair β-cell compensatory mechanisms also could act in concert with genetic defects in insulin action to cause diabetes.

Table 2
Clinical Phenotype of Maturity-Onset Diabetes of the Young (MODY) Subtypes

Type of MODY	Diabetes	Complications	Therapy	Other
1	Severe	Similar to late-onset type 2 diabetes mellitus	Oral hypoglycemic agents, insulin	——
2	Mild	Rare	Diet, oral hypoglycemic agents	Low birth weight
3	Severe	Similar to late-onset type 2 diabetes mellitus	Oral hypoglycemic agents, insulin	Low renal gluccose threshold?
4	Moderate	Not determined	Diet, oral hypoglycemic agents	——
5	Severe	Nephropathy	Insulin	Renal dysfunction and cysts

Reprinted with permission from Stoffers DA. In: *Current Review of Diabetes,* Simeon Taylor. Philadelphia, Pa: Current Medicine 1999:11-21.

POSSIBLE ROLE OF GENETIC SCREENING

Genetic screening for specific mutations in diabetes genes may offer several therapeutic advantages.[5] Determination of the genetic mutations in cases of MODY may assist in the determination of prognosis (Table 2), choice of optimal therapy, and early implementation of the appropriate lifestyle to reduce complications. Although all forms of MODY identified so far are characterized by an insulin secretory defect, the precise nature of the defect and the clinical course vary according to the genetic defect(s). For example, MODY2 is characterized by mild fasting hyperglycemia that is often already evident in early childhood. However, less than half of the patients will progress to overt diabetes, few will develop complications, and most will not require medical intervention, except during pregnancy. These characteristics allow a clinical approach of limited monitoring. As another example, the clinical phenotypes of MODY1 and MODY3 are both characterized by progressive deterioration of glucose homeostasis, with some subjects remaining well controlled on diet or sulfonylureas and others progressing to insulin therapy. The incidence of complications for MODY1 and MODY3 resemble those of late-onset type 2 DM. MODY5 appears to be particularly associated with renal complications and cysts. Genetic screening can be an important predictor of both quality and quantity of life (Table 2) and the need for more rigid therapy than in MODY2 patients. Therefore, childhood diabetes is a specific instance in which genetic screening can be helpful. Several studies have now attributed previously diagnosed type 1 DM (usually autoimmune) to mutations in *HNF-1α (MODY3).*[6,7] These subjects were not characterized by the expression of autoimmune markers but were given the diagnosis of type 1 DM because of the early age of onset. This is significant, as the future clinical course is distinctly different for type 1 DM and type 2 MODY. Screening and diagnosis of MODY provides a more measured approach in terms of clinical therapy, especially involving insulin. Routine screening in the clinical setting, however, will require ongoing and future technological advances in mutation detection before it can become practical and economically feasible.

FUNCTION AND IDENTIFICATION OF MODY GENES

A brief review of glucose metabolism, glycolysis, and insulin secretion provides a foundation for comprehending the function and identification of MODY genes.[2,3] The islet β cell is uniquely equipped to sense blood glucose concentrations and to secrete insulin in a precise

fashion to maintain glucose in a narrow physiologic range.[8] In the pancreatic β cells and in hepatocytes, uptake of glucose is mediated by the high K_m glucose transporter GLUT2. Glucose metabolism must be initiated to stimulate insulin secretion. Glycolysis is the first step in glucose metabolism and occurs in both the pancreas and liver. Glucokinase, the low K_m rate-limiting hexokinase that phosphorylates glucose to glucose-6-phosphate, is the catalyst. Glycolysis in the β cell results in the generation of adenosine triphosphate (ATP), which causes the closure of ATP-sensitive potassium channels. This depolarizes the β cell, causing calcium channels to open. Calcium then flows into the cells, triggering secretion of insulin.

The first MODY locus to be identified *(MODY2)* was found to encode glucokinase, the key regulatory enzyme in glucose metabolism (Table 3).[9,10] Interestingly, mutations in the glucokinase *(MODY2)* gene not only produce DM but also can produce reduced birth weight in the fetus.[11] Most mutations in the glucokinase gene decrease insulin secretion and appear to cause diabetes through a gene-dosage effect. Interestingly, there is at least 1 activating mutation that causes hyperinsulinemia and hypoglycemia,[12] further establishing the critical function of glucokinase in sensing blood glucose levels and in maintaining a normal glucose-induced secretory insulin response. This may reflect decreased fetal insulin, which functions as a growth factor in utero. Glucokinase mutations in the fetus may impair fetal insulin secretion in response to normal maternal glucose levels. If, however, the mother also is heterozygous for the glucokinase mutation, the higher maternal glucose levels will provoke greater fetal insulin secretion, leading to MODY2 infants with normal birth weights. Other characteristic features of MODY2 include the mildness of the disease compared with MODY1, MODY3, and MODY5; the multiple variants of mutations found (~50); and the rarity of complications.

All of the MODY genes[1,3,4] except that for MODY2 encode transcription factors localized to the pancreas and other tissues such as liver and kidney (Table 3). Two approaches, positional cloning and screening of candidate genes, resulted in the identification of specific transcription factor mutations. Positional cloning using the previously characterized large MODY pedigrees led to the identification of *MODY1* and *MODY3* as the genes encoding hepatocyte nuclear factors *HNF-4α* and *HNF-1α,* respectively. The HNFs were originally discovered as a heterogeneous family of transcription factors that control liver-specific gene expression. Subsequently, HNFs were identified in other tissues, including pancreatic islets.[13] The HNFs form a network of cross-regulatory transcription factors that regulate expression of genes involved in a wide range of cellular processes in metabolism, but often are equally important in differentiation and development.

The most extensive and well-characterized MODY pedigree is the RW pedigree, which has been followed prospectively since 1958; it now consists of 455 members in 7 generations and includes 74 diabetics.[14] Diabetes in the RW pedigree was linked to a DNA polymorphism on chromosome 20 in 1991.[15] Once *HNF-1α* was determined to be the *MODY3* gene, a scan of known genes in the *MODY1* interval raised *HNF-4α* as an intriguing candidate, since HNF-4α is a known upstream regulator of *HNF-1α*. This hypothesis was confirmed with the identification of the Q268X nonsense mutation in the RW pedigree.[2] The relationship between *HNF-1α* and *HNF-4α* in diabetes is further underscored by the identification of a MODY mutation in the *HNF-4α* promoter in an Italian MODY pedigree.[16]

HNF-4α encodes an orphan member of the nuclear hormone receptor superfamily that regulates gene expression required for glucose transport and metabolism. The Q268X mutation results in the synthesis of a truncated protein that does not activate gene transcription.[17] Mutations in *HNF-4α* remain a relatively rare cause of diabetes as only 6 mutations have been reported. Interestingly, 1 of these mutations (V3931) was identified in a family with late-onset type 2 DM, demonstrating the overlap that different mutations provide for MODY and the adult phenotype of type 2 DM.

Table 3
MODY Genes

MODY	Chromosome	Mutated Gene	Gene Product	Distribution	Regulatory Function
1	20q	Hepatocyte nuclear factor *(HNF)-4α*	Transcription factor, nuclear hormone receptor family	Islets, liver, kidney, intestine	Glucose transport and metabolism genes; HNF-1α gene expression
2	7p	Glucokinase *(GK)*	Enzyme	Islets, liver	Glucose phosphorylation
3	12q	Hepatocyte nuclear factor *(HNF)-1α*	Transcription factor, POU homeodomain	Islets, liver, kidney	Insulin, glucose transport and metabolism genes
4	13q	Insulin promoter factor-1 *(IPF-1)*	Transcription factor, Antp homeodomain	Islets, duodenum, stomach	Pancreas development, β-cell gene expression
5	17-cenq	Hepatocyte nuclear factor *(HNF)-1β*	Transcription factor, POU homeodomain	Islets, liver, kidney	Dimerization partner for HNF-1α

Reprinted with permission from Stoffers DA. In: *Current Review of Diabetes*. Simeon Taylor. Philadelphia, Pa: Current Medicine 1999:11-21.

The identity of the *MODY3* locus also was determined by a positional cloning approach. In 1996, a cytosine insertion was identified in codon 291 of *HNF-1α* (P291fsdelC). This segregated with DM in a MODY3 pedigree. This particular mutant of the *HNF-1α* gene is a frameshifted truncated protein that appears to function in a dominant negative manner.[18] Mutations (57 to date) in *MODY3* are highly prevalent, accounting for 64% of MODY in the United Kingdom, 30% of early-onset type 2 DM in Germany, and 15% to 20% of MODY in Japan. The P291fsdelC mutation has appeared in at least 9 distinct haplotypes in Germany, Britain, the United States, Sweden, and Japan, implying the existence of a mutational hot spot. HNF-1α appears to regulate transcription of the insulin gene, the *GLUT2* gene, and other genes encoding components of the β-cell glycolytic pathway.

The most recently described MODY gene *(MODY5)* encodes HNF-1β, another member of the transcriptional regulatory network that includes HNF-1α and HNF-4α (Table 3). The *HNF-1β* gene was screened because it was known that HNF-1β can function as a heterodimerization partner with HNF-1α. A nonsense mutation in *HNF-1β*, R177X, was identified in a small Japanese pedigree with early onset of DM at age 10 and 15, but one member developed DM later, at age 40.[12] All had evidence of diabetic neuropathy. Subsequent screens have identified 2 additional mutations in families with early-onset DM and MODY associated with renal failure and renal cysts, and another mutation in late-onset type 2 DM not associated with kidney disease.

The identity of the *MODY4* locus reflects the close relationship between *pancreatic development* and glucose homeostasis. Experimental gene inactivation in mice is uncovering a growing number of transcription factor genes whose normal expression is required for full development of the exocrine and endocrine pancreas (Table 4). These genes are being evaluated as candidate diabetes genes. The first positive example of this approach came from mice with homozygous disruption of the *IPF-1* gene and resultant total pancreatic agenesis. IPF-1, a homeodomain transcription factor, is implicated in the transcriptional regulation of key β-cell genes. In humans, the first MODY4 family was discovered when a rare subject with pancreatic agenesis was found to be homozygous for an inactivating cytosine deletion mutation in the protein coding sequence of *IPF-1* (Pro63fsdelC).[19] Subsequently, the heterozygous mutant allele within both branches of the extended family of the proband was linked to MODY.[8] Three members of this pedigree satisfy the strictest criteria for the diagnosis of MODY, and 2 additional heterozygous subjects developed diabetes or glucose intolerance by 30 years of age, thus establishing *IPF-1* as the MODY4 gene. To date, at least 7 additional heterozygous *IPF-1* mutations have been discovered in approximately 5% to 6% of familial late-onset type 2 DM patients in France and the United Kingdom and in a small number of additional MODY pedigrees.

Table 4
Transcription Factor Gene Knockouts and Pancreas Development

Factor	Knockout Mouse Phenotype
IPF-1	Pancreatic agenesis
Pax-4	Decreased β and δ cell numbers
Pax-6	Decreased α cell numbers
Isl-1	Dorsal pancreatic agenesis
Beta2/NeuroD	Decreased β-cell numbers
Nkx2.2	Impaired β-cell differentiation, no insulin synthesis
Nkx6.1	Decreased β-cell numbers
p48	Pancreatic agenesis; ectopic islet cells in the spleen

Reprinted with permission from Stoffers DA. In: *Current Review of Diabetes*, Simeon Taylor. Philadelphia, Pa: Current Medicine 1999:11-21.

THE FUTURE

While the recent advances in MODY genetics have been most exciting, there remain additional MODY genes to be uncovered. In 2 MODY populations in France and England, in which screening for possible mutations in all 5 MODY genes has been undertaken, the genetic defect in 16% to 20% of MODY pedigrees remains a mystery. An ever-increasing number of genes whose normal function is required for full development of the pancreas are being identified through analysis of the phenotypes of knockout mice (Table 4).[20] Some of these genes probably will turn out to play a role in human type 2 DM. In support of this concept, several mutations in the human *BETA2* gene were recently reported in familial late-onset type 2 DM.[21] Other members of the transcriptional regulatory network of hepatocyte nuclear factors also are logical candidate diabetes genes to consider.

Most MODY subjects exhibit decreased insulin secretion and lean body mass. However, there are other MODY pedigrees (in which mutations in *MODY1,*

MODY2, and *MODY3* have been ruled out) that include diabetics with high circulating insulin levels and also a higher incidence of obesity than their unaffected relatives.[22] This form of MODY may be caused by mutations in a distinct class of genes whose function is not specifically involved in the regulation of β-cell development and function.

CONCLUSION

MODY is an autosomal dominant monogenic form of type 2 DM that is characterized by a primary defect in insulin secretion. Four of the 5 MODY genes discovered to date encode transcription factors that regulate β-cell development and function. This observation has transformed our concept of diabetes into a disorder of the β cell and has intensified research efforts aimed at improving the function and mass of insulin-producing β cells. Additional MODY genes remain to be uncovered. The identification of mutations in MODY genes in common late-onset type 2 DM indicates that MODY is a useful paradigm for type 2 DM and that a genetically programmed impairment of the β cell may underlie a greater proportion of type 2 DM than previously suspected.

REFERENCES

1. Tattersall RB, Fajans SS. *Diabetes* 1975;24:44-53.
2. Stoffers DA. *Current Review of Diabetes*, Simeon Taylor. Philadelphia, Pa: Current Medicine 1999:11-21.
3. Froguel P, Velho G. *Trends Endocrinol Metab* 1999;10:142-146.
4. Hattersley AT. *Diabetes Med* 1998;15:15-24.
5. Matyka KA, et al. *Arch Dis Child* 1998;78:552-554.
6. Miura J, et al. *Diabetes Res Clin Pract* 1997;38:139-141.
7. Kawasaki E, et al. *J Clin Endocrinol Metab* 2000;85:331-335.
8. Matschinsky F. *Diabetes* 1996;45:223-241.
9. Hattersley A, et al. *Lancet* 1992;339:1307-1310.
10. Froguel P, et al. *Nature* 1992;356:162-164.
11. Hattersley AT, et al. *Nat Genet* 1998;19:268-270.
12. Horikawa Y, et al. *Nat Genet* 1997;17:384-385.
13. Miquerol L, et al. *J Biol Chem* 1994;269:8944-8951.
14. Fajans SS, et al. *Molecular Pathogenesis of MODYs*, Karger. New York, NY; Frontiers in Diabetes. 1998;15:13.
15. Bell GK, et al. *Proc Natl Acad Sci USA* 1991;88:1484-1488.
16. Gragnoli CT, et al. *Diabetes* 1997;46:1648-1651.
17. Stoffel M, Duncan SA. *Proc Natl Acad Sci USA* 1997;94:13209-13214.
18. Yamagata K, et al. *Diabetes* 1998;47:1231-1236.
19. Stoffers DA, et al. *Nat Genet* 1997;15:106-110.
20. Habener JF, Stoffers DA. *Proc Assoc Am Phys* 1998;110:12-21.
21. Maleci MT, et al. *Nat Genet* 1999;23:323-328.
22. Doria A, et al. *Diabetes Care* 1999;22:253-261.

Genetic Biotechnology and Patent Rights

William D. Noonan, MD, JD
Klarquist Sparkman, LLP
Portland, Oregon

INTRODUCTION

As the Human Genome Project (HGP) nears completion, scientists have engaged in a spirited discussion about ownership of genetic information and to what extent it should be patentable. Billions of dollars of private investment have contributed to cloning DNA sequences and protecting biotechnology resulting from this research, such as genetic tests. Many private companies have attempted to broadly protect intellectual property rights by patenting total or partial gene sequences and the products these sequences encode. Many of the patents obtained on gene sequences and related biotechnological inventions have been and remain controversial.

Just as the HGP promises to transform the future practice of medicine, the associated legal developments signal a transformation in medical economics. In this article the historic perspective of this topic is succinctly presented, and the legal requirements for patentability are discussed. This article also reviews recent controversies about patenting genomic sequences, partial gene sequences, and genetic tests, and the patent rights of a patient when biological inventions are made from his own tissues. The results of several recent patent infringement lawsuits also are summarized. In the conclusion section, public policy issues about patenting genetic technologies are discussed.

HISTORICAL PERSPECTIVE

The controversy about patents dealing with genetic inventions is only the latest chapter in a long and uncomfortable history of patents in medicine. At the *beginning of the 20th century,* the US Patent Office was reluctant to grant patents on medical inventions because medicine was considered too unscientific for its inventions to deserve the imprimatur of a patent. Conversely, many physicians considered patents unethical because medicine was an altruistic calling that was inconsistent with anything as commercial as a patent.

These attitudes changed in the *mid-20th century* when medicine developed a more scientific basis. During the golden age of pharmacology in the 1940s and 1950s corporations began to spend millions of dollars developing new drugs. Patent protection was needed to prevent competitors from taking unfair advantage of the expensive experimental work of research-based corporations. The attitude of practitioners in organizations such as the American Medical Association (AMA) also evolved, and

Letter From the Editor

This lead article is different than most in *GROWTH, Genetics, & Hormones.* The relationship of patenting medical devices, drugs, diagnostic procedures, and genes to the practice and economics of medicine is knowledgeably presented by Dr. Noonan, Doctor of Jurisprudence (1980) and Doctor of Medicine, magna cum laude (1994), Resident in Medicine and Ophthalmology, Professor of Law and Medicine, recipient of many outstanding awards as physician and as lawyer, former member of the National Board of Medical Examiners, and a recurrent witness in Washington, DC, and in Portland, Oregon, where he lives and is a patent lawyer.

Robert M. Blizzard, MD

by *mid-century* the AMA changed its canon of ethics to allow patenting of most medical inventions. Inventors of medical devices and pharmaceuticals began to take advantage of the rewards offered by patent protection, which allowed them to prohibit others from using a patented invention without paying a royalty to the patent owner.

A revolution in patent law occurred in the 1970s, when Boyer and Cohen obtained their basic patents on recombinant DNA techniques. In 1980, the US Supreme Court dramatically expanded the scope of patent law by permitting the patenting of living, genetically modified organisms. The importance of patents in the life sciences also was increased by the Bayh-Dole Act of 1980, which granted patent rights to universities. Universities could hold title to patents developed with federal research grant money. Much of this research was related to medical and biological technologies, and many early biotechnology companies such as Genentech, Inc. were founded using university technology that had been patented.

Following these changes in the law, the US Patent and Trademark Office (PTO) was inundated with hundreds of thousands of patent applications on biological inventions during the 1980s. The PTO began to issue patents on a broad spectrum of such inventions, including (1) recombinant DNA and proteins, (2) transgenic animals, (3) research tools such as the polymerase chain reaction (PCR), (4) new cell lines, (5) enzymes and probes, (7) gene sequences, and (8) mutations associated with disease, such as polymorphisms seen in cystic fibrosis or muscular dystrophy.

REQUIREMENTS FOR PATENTABLE INVENTIONS

Genetic patents puzzle some scientists, who wonder how a patent can be issued on something that already exists, such as a naturally occurring DNA sequence or a protein product of a gene. The explanation is outlined in Table 1. Things occurring in nature that are converted into something that does not exist in nature are patentable, if they are sufficiently nonobvious. A DNA sequence is not patented as it occurs in nature, but it can be patented in a purified, isolated, or synthetic form that is useful in a laboratory or that can be introduced into a vector for gene delivery or for in vitro protein production. Since a laboratory form of DNA does not exist in nature, it is "new" in the sense required by the patent law. Moreover, if the sequence of an unknown gene is not predictable in advance, it cannot be said to be obvious in the legal sense and is, therefore, patentable.

PATIENTS' RIGHTS IN PATENTED TISSUE AND CELLS

The last 2 decades have seen unceasing controversy about genetic and other biological patents. An early case was Moore vs University of California, which concerned a patient (Moore) who was treated for hairy cell leukemia at the University of California. Moore's spleen was removed as part of his treatment. The spleen cells were found to produce large amounts of lymphokines, which were potentially commercially valuable. The spleen cells were immortalized, patented, and licensed to a biotechnology company. Moore then found out that his physicians and the university had used his biological tissue for their own profit.

Moore sued, asking to be named as a co-inventor on the patent, asserting that the cells were his property. The Supreme Court of California decided in 1990 that a patient *does not* become a co-inventor by donating tissue, nor does he own the cells once they are taken from his body. However, Moore still won because the Court found his physicians failed to obtain a fully informed consent. Physicians and researchers must tell a patient if tissue taken from their body is to be used for potentially profitable research. Damages can be collected from physicians or researchers who fail to obtain informed consent from a patient whose tissue is used in research.

PATENTS RELATED TO GENOMIC SEQUENCES AND PARTIAL GENE SEQUENCES

Perhaps the greatest controversy about patents in biotechnology has been the furor over patenting isolated nucleic acids having sequences that are found in the

Table 1
PATENTING GENES AND PROTEINS

- Must be new, useful, and nonobvious
- Product of nature patentable if converted to a new form
  - –Purified or synthetic DNA
  - –Coupled to a nonnative promoter
  - –Inserted in a vector

human genome. Patents have been issued for many years on purified, synthetic, or isolated DNA sequences that encode proteins of medical or other biological interest. The DNA sequence patents are considered important because they protect the "factories" that produce recombinant proteins. In the absence of strong patent protection at the molecular level, private investment in new technologies is diminished. Patents on genomic DNA sequences also protect techniques of molecular diagnosis, such as the detection of polymorphisms associated with disease. This protection is considered important by companies developing test kits for detecting genetic diseases.

As genomic information has become available from the HGP and elsewhere, isolated or purified nucleic acid molecules containing new genetic sequences have been patented on a large scale. For example, Celera Genomics Corporation has filed patent applications on many thousands of isolated partial gene sequences and expressed sequence tags (ESTs), even though a complete gene sequence or the biological significance of the sequence is unknown. The PTO has issued patents on such purified or synthetic "new" sequences. These are considered "nonobvious" because the sequences could not be predicted in advance. Purified EST molecules also were considered to satisfy the "useful" requirement for patentability because they could be used as gene probes to find the full gene from which the EST was derived.

Since the PTO has issued patents on multiple ESTs that map to a single gene, many different patents often exist that protect a partial gene sequence before the complete gene is sequenced and before the biological significance of the gene product is determined. This aberration has upset the balance of innovation because anyone who wants to work with the completed gene may have to obtain a patent license from 5 or 6 different patent holders who have staked a patent claim to different portions of the gene sequence. This situation acts as a disincentive to researchers and to companies that do the hard work of fully sequencing a gene and determining its biological function.

The PTO has recently changed its policy about protecting fragments of DNA sequences of unknown function such as ESTs. In March 2000, the PTO issued new guidelines that now require that a patentable DNA molecule have a particular and more substantial use than merely acting as a probe for use in further research. Moreover, possession of a partial DNA sequence such as an EST will only entitle one to patent a molecule that contains the short sequence itself, and not a longer DNA molecule such as a gene or cDNA that includes the shorter EST sequence. Hence, even if a patent is obtained on an EST, anyone who eventually sequences the entire gene and determines its function will not have to obtain a patent license from the EST patent owner to work with the full gene. These policy changes will likely limit some of the more egregious instances of DNA patent abuse.

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for internists, pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Drug companies and researchers also are making DNA sequence information available through public databases as quickly as it is known. It then becomes "prior art," which can be used to prevent subsequent patenting of nucleic acids that contain the sequence, or at least prevent others from subsequently obtaining broad patent protection on related gene sequences.

PATENTS ON GENETIC TESTS

Patents on the development of laboratory tests that permit the detection of a genetic disorder also have been controversial. For example, Myriad Genetics holds patent rights on genetic tests that detect mutations in the gene *BRCA1*. When present, these mutations predispose to the development of breast and ovarian cancer. Some university researchers complained that the $2,500 fee charged by Myriad for each test was so high that it precluded them from continuing research about the gene and its mutations. Although the test fee was subsequently reduced, such high charges may deter unfettered research and can create obstacles to medical advances.

However, a fair discussion of genetic patents requires that both the benefits and burdens be considered. A benefit is that biotechnology patents have attracted billions of dollars of private research into genetic and molecular biology research, which ultimately advances patient care. Patents are essential tools to biotechnology companies, as evidenced by substantial changes in the companies' stock prices in response to news about their patents. The importance of patents to biotechnology companies was reflected in the collapse of biotechnology stocks in mid-March of this year (2000) after President Bill Clinton and Prime Minister Tony Blair made a joint statement that seemed to question whether DNA molecules should be patentable. President Clinton subsequently clarified that he was questioning only whether raw genetic information should be patentable. However, genetic information per se is not, and has not been, patentable; only purified molecules that include the sequence are patentable subject matter.

Although genetic patents do increase the cost of genetic tests, the billions of dollars of investment capital they have attracted to genetic research promise to transform medicine quickly, and move genetics to a central role in medical practice. Just as pharmaceutical patents provided the impetus for the transformation of medicine in the *mid-20th century,* biotechnology patents will very likely provide the economic incentives for the practice of widespread molecular medicine *in the 21st century.* Private companies are already developing high-production genomic techniques for large-scale genetic diagnosis by using sophisticated software technologies and cDNA microarrays. Such technologies should quickly move genetic practitioners and researchers to an even more important role in medical practice.

GENETIC PATENT LAWSUITS

Several high-profile patent infringement cases concerning DNA patents illustrate the importance of "genetic" patents. Recently, the University of California, San Francisco sued Genentech, Inc. concerning its patented sequence of cDNA, which included a coding sequence of hGH. Genentech, Inc. allegedly used the hGH sequence to produce rhGH. Genentech, Inc. settled out of court by paying a large amount to UCSF for the alleged violation of UCSF's patent rights.

In another suit, the University of California accused Eli Lilly of infringing its patent on recombinant mammalian insulin. However, the court in this case found the patent invalid. UC scientists had determined the cDNA sequence of the gene for preproinsulin and proinsulin only in *rats.* The court held that the patent as written was invalid because only the *rat* gene sequence was disclosed in the patent application. Since the scientists had determined the sequence only in the rat, the patent was invalid for attempting to also protect all *mammalian* sequences (including the human sequence that had not yet been determined).

CONCLUSION

Patents concerning biotechnology are controversial but necessary. They are designed to prevent others from making, using, or selling the patented invention without permission. Their purpose is to reward inventors and investors and to encourage private investment in technology development. The broader interests of society also are protected by limiting the effective life of a patent to 20 years from the day the application is filed, after which time the technology is available for all to use. Typically, gene patents are used to cover items listed in Table 2.

Table 2
Typical Gene Patent Items Covered

- Purified protein (if novel)
- DNA (*ORF)* encoding the protein
- rDNA operably linked to a promoter
- Cell transformed with the nucleic acid
- Transgenic animal into which the transgene is introduced
- Oligonucleotides (probes) of 20, 30, or 50 contiguous nucleotides; antisense oligonucleotides
- DNA that hybridizes to the sequence (includes variants)

Patent rights usually are decided in courts of law. The actual outcomes of court cases concerning biological patents illustrate, in my opinion, that legislative changes are only occasionally necessary to curb perceived problems with patents. Currently, courts construe genetic patents very narrowly and invalidate them if the patent has been written too broadly. The legal stringency courts have applied to patents recently has prompted the PTO to examine biotechnology patent applications much more diligently before issuing a patent.

The public policy challenge of the coming years will be to find a beneficial balance between the need to encourage private investment in molecular medicine with reasonable patent rights, while not unduly limiting the ability of researchers to rapidly advance new discoveries within medical science. Some professional organizations, such as the American College of Medical Genetics (ACMG), have issued policy papers stating that genes and mutations are naturally occurring substances that should not be patented **(http://www.faseb.org/genetics/acmg/pol-34)**. If implemented, such an extreme position could undermine the biotechnology industry, drive private capital out of the field, and greatly slow the progress of molecular medicine.

A more moderate position was taken by the American Society of Human Genetics (ASHG) **(http://www. faseb.org/genetics/ashg/policy/pol-08)**, which did not oppose the patenting of nucleic acid molecules that were found to code for therapeutic proteins or could be used as disease gene probes for specific diagnostic tests. However, ASHG did oppose patenting EST molecules because they were only tools for further research and therefore lacked "patentable utility." ASHG also was concerned that EST patents would create a morass of competing patents for the same DNA molecule when its full sequence was eventually determined. ASHG subsequently commended the US PTO **(http://www. faseb.org/ashg/policy/pol-39)** for the new guidelines it adopted in March 2000, which will make it much more difficult to patent EST molecules that are only tools for further research.

Recent court decisions and changes in PTO policy have raised the standards for patentability of nucleic acid molecules and have addressed many of the objections to the broad scope of earlier nucleic acid patents. These changes should help overcome many of the more reasonable objections to patents dealing with biotechnology and allow molecular medicine to continue to develop rapidly with the economic protection provided by patents.

Gender Assignment and Reassignment in 46,XY Pseudohermaphroditism and Related Conditions: A Commentary

Meyer-Bahlburg discusses the current intense debate in managing patients with intersexuality. Three major issues are the focus of the presentation: (1) gender assignment, (2) indications for genital surgery (particularly in the newborn period), and (3) the disclosure of medical information to the patient. The pertinence of these considerations was precipitated in part by Diamond's and Sigmundson's published guidelines (*J Sex Res* 1997;34:199-211, and *Arch Pediatr Adolesc Med* 1997;151: 1046-1050). These guidelines strongly recommend assigning all 46,XY patients who incurred penile loss, micropenis, androgen insensitivity stages 2 and 3, hypospadias, 5α-reductase deficiency, or 17,β-OH steroid dehydrogenase deficiency to the male sex. The Intersex Society of North America, primarily consisting of adults with intersex problems dating to infancy, also has published recommendations that center on the avoidance of genital surgery without the patient's informed consent, unless it is absolutely necessary for the physical health and comfort of the intersex child.

Meyer-Bahlburg appropriately and wisely states, "to evaluate such recommendations we have to place them in historical perspective." He proceeds to do so, and starts with consideration of the gender question and how we have increased our knowledge since the early 20th century so that we now are aware that *nature* (the influence of male hormones in utero on imprinting the brain along male lines) is an important phenomenon, particularly in determining gender role and, to a lesser extent, gender identity. He emphasizes the significant difference in effect, however, among species (eg, guinea pigs and humans). He also emphasizes that much data support the role that *nurture* plays, meaning the effect of environment resulting from early sex assignment to a newborn who has the genitalia to function in the sex of assignment. He stresses that evidence from long-term follow-up of intersex patients themselves will be the final arbiter of the adequacy of a given management policy, and that this evidence is extremely limited, especially in the case of male pseudohermaphroditism. The very few scattered cases that are discussed in great detail in the media and public arena are inadequately documented in sufficient psychological detail to determine an absolute management recommendation. Meyer-Bahlburg describes in detail his reasons for reservations in the John/Joan case, maintaining that *nature* was proven to be the decision-making factor in a male child with ablated penis who was raised as a girl (Joan) from 21 months and then elected to return to a male role at 14 years (John). Meyer-Bahlburg appropriately states, "To move forward in this difficult area, we must carefully distinguish between conclusions for which we really have good evidence and statements based on interpolation." Meyer-Bahlburg summarized that the evidence available to date permits only tentative policy-relevant conclusions, which are:

- The organizational effects of prenatal androgens are more noticeable in gender role (behavior) than in gender identity.
- Gender identity can develop as female or male over wide variations of gender role (behavior).

- The majority of 46,XY intersex patients seem to develop an identity commensurate with the assigned gender and do not change their gender later.

- Gender identity assigned in childhood usually will continue into adolescence and adulthood, but patient-initiated gender change in intersex patients seems to happen more often at those times. Thus, long-term follow-up into mid-adulthood is essential if one wants to arrive at definitive conclusions concerning the appropriate way to assign intersex patients at birth.

- More female-assigned 46,XY patients initiate gender change to male than male-assigned 46,XY patients initiate gender change to female. There is suggestive but not conclusive evidence that this is more frequent in patients with a history of fully male-typical prenatal androgenization.

- There is, at this stage of research, no unambiguous evidence for or against female gender assignment of 46,XY patients, even in the prenatally most androgenized conditions.

- The number of well-documented cases, especially regarding prevalence rates of gender change, is uncomfortably small to draw definitive conclusions, and psychological details and assessment measures often leave much to be desired.

- The only way of obtaining a sufficient empirical basis for an intersex management policy is to conduct sophisticated comprehensive psychological follow-up studies with reasonable sample sizes. This will require collaboration among clinics.

Editor's comment: *The issue of the appropriate assignment of a child with a 46,XY karyotype and ambiguous genitalia remains difficult, as evidenced by the controversies and accusations made by individuals and by societies. Meyer-Bahlburg brings a much needed calm, nonemotional scientific approach for consideration.*

The nature and nurture effects on gender identity and gender role are both important and can be compared with the inclusive roles of the gene versus environment on behavior that were in controversial consideration in the early and mid 20th century. The approach of a recently organized North American Task Force on Intersexuality (E-mail: perkos@musc.edu), which consists of a group of pediatric urologists, endocrinologists, other physicians, psychologists, and others, may produce a scientific forum to enhance our data-gathering capability regarding the outcomes of sex assignments made in the past to children who now are adults. Gathering the data will enhance our capability to make the humane decisions that we all wish to do for our patients. Importantly, in medicine we must remember that there usually are those who suffer as a result of any specific untried medical approach to save lives even though the treatment ultimately proves beneficial. Examples are kidney, heart, and lung transplants, the administration of human GH, and the administration of blood at surgery to prevent death. All of these in the early stages of their use were associated at times with life-threatening hepatitis or AIDS viruses. We as physicians and concerned humans have great empathy for those who suffer as a consequence, but that does not mean that great benefit will not come to many as a result of deliberately pursuing the truth. Personally, I thank Dr. Meyer-Bahlburg and others like him who are pursuing a calm scientific perspective to solve a complex problem.

What Causes Low Rates of Childbearing in Congenital Adrenal Hyperplasia (CAH): A Commentary

Much has been written concerning this topic over many years. In the present article Meyer-Bahlburg has thoughtfully reviewed the etiologic considerations. The purpose was to revisit the issue, review the status of the empirical evidence —especially the role of behavioral determinants—and suggest additional hormone-related psychological factors that may contribute to the low fertility rates of women with congenital adrenal hyperplasia (CAH). The possible anatomic and psychological factors contributing to the overall reduction of fertility in women with classic CAH are considered initially. A consistent observation has been the predominant occurrence of low birth rates in women with salt-wasting (SW) CAH compared with those with simple virilizing (SV) CAH. Reduced heterosexual activity certainly is a contributing factor, which stems from several causes. Meyer-Bahlburg has reviewed all of these eloquently. However, the limitations of studies pursued in these areas are many. Despite these, the evidence clearly indicates that the reduced fertility of women with classic CAH has a variety of other reasons. Nonoptimal hormonal control remains a major reason. Ovulatory failure secondary to steroid excess is an important barrier to conception in many CAH women. Considerable evidence exists suggesting that the steroid excess is not just an outcome of corticotropin oversecretion. Other contributing factors appear to be (1) a mild degree of corticotropin hyperresponsiveness to corticotropin-releasing hormone; (2) altered enzyme kinetics, including reduced catalytic efficiency of the mutated 21-hydroxylase enzyme with resulting increases in the precursor hormones progesterone (P) and 17-hydroxyprogesterone (17OH-P) even in the presence of excess glucocorticoid administration; (3) overactivation of the renin-angiotensin-aldosterone axis with ensuing stimulation of adrenocortical biosynthesis; and (4) alterations of the hypothalamic-pituitary-ovarian axis, as indicated by abnormal gonadotropin dynamics, polycystic ovaries, and excessive ovarian production of P, 17OH-P, and androgens. Consequently, new combinations of treatments that go beyond mere corticotropin suppression are being developed to improve the overall quality of hormonal control in CAH.

Meyer-Bahlburg also discusses the possible role that dexamethasone administration to mothers pregnant with CAH female infants might play in enhancing the number of births in these CAH females when they become adults, as such

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
SCHOOL OF MEDICINE

GROWTH, Genetics, & Hormones
Volume 16, Number 3

Post-Program Self-Assessment/CME Verification
Answer Sheet

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Answer each of the questions or statements contained in the Post-Program Self-Assessment/Course Evaluation. There may be more than one correct answer. Participants are encouraged to refer to the related articles for evaluation of their responses. To receive Category 1 credit, complete the self-assessment exam/course evaluation and record your responses on the answer sheet. Enclose a check for $20 made payable to the University of Virginia or complete credit card information. Mail the answer sheet and fee to:

GGH
PO Box 40
Garwood, NJ 07027-0040

A certificate will be mailed to you. This enduring material was approved for credit November 2000. Eligibility for credit expires November 2003.

Name (print clearly) Degree Specialty

Street

City State Zip

Phone SS No.

Amount enclosed: _____ ❑ Check

❑ Credit Card (circle one) VISA MC

Credit Card Number: _____ Exp Date: _____

Signature (credit cards only): _____

Record your answers by circling the appropriate letter(s) for each question.

1. a b c d
2. a b c
3. a b c d e
4. a b

Course Evaluation

5 = Excellent 4 = Above average 3 = Good 2 = Below average 1 = Poor

Please evaluate this course with respect to the following:

1. Educational value of newsletter	5	4	3	2	1
2. Clinical relevance of articles	5	4	3	2	1
3. Newsletter style/format	5	4	3	2	1
4. Length of articles	5	4	3	2	1
5. Were the educational objectives met?	5	4	3	2	1

6. In your opinion, how could this newsletter be improved?

_____

_____

_____

treatment has the potential to minimize the physical and mental alterations so often seen in virilized CAH female newborns.

Meyer-Bahlburg HFL. *J Clin Endocrinol Metab* 1999;84:1844-1847.

Editor's comment: *The lead article in the December 1999 issue of* GGH *(15:3:33-41) was entitled* Adult Consequences of Pediatric Endocrine Disease, I: Congenital Adrenal Hyperplasia. *The section entitled* Pregnancy in CAH Females *reads as follows:*

> *The incidence and prevalence of pregnancy in SWCAH females is very low compared with that in SVCAH (simple virilizing CAH) patients. Mulaikal et al reported on 80 adult female CAH patients (40 with SWCAH and 40 with SVCAH). Twenty-five pregnancies were reported among 15 women with SVCAH, but only 1 pregnancy was recorded in 40 patients with SWCAH. In the Cardiff experience of 16 patients (11 SWCAH, 5 SVCAH), a 40% ovulation rate, as measured by salivary progesterone, was found. Three of 5 patients with SWCAH and 2 of 3 patients with SVCAH who had both an adequate introitus and were sexually active produced 8 pregnancies. This study highlights the potential for improved fertility in compliant patients who are treated early, who have adequately reconstructed genitalia, and who are followed closely during pregnancy for progesterone, 17α-hydroxyprogesterone, and testosterone levels. A 1998 review by Garner (*Semin Perinatol *1998;22:446-456), entitled "CAH in Pregnancy," provides an overview of CAH in both mothers with and without CAH and in potential CAH fetuses in utero. More information on the frequency of pregnancy in SWCAH is very much needed, but the pregnancy rate certainly is low from all data presented to date.*
>
> *What are the possible causes of the difference in pregnancy rates in SVCAH and SWCAH? The first possibility is that therapeutic noncompliance is greater among SWCAH patients. The second possibility is that there is a higher frequency of menstrual irregularity among SWCAH patients, reflecting less ovulation in this group. Third, there is a lower incidence of marriage in the SWCAH patients than in the SVCAH patients and, therefore, there is less opportunity for pregnancy. Fourth, the vaginal introitus is more frequently inadequate in the SWCAH group compared with the SVCAH group (53% vs 18%). Another possibility is that SWCAH patients engage in heterosexual activity less frequently.*
>
> *The necessity for cesarean section in patients with SVCAH is very high. Of the 15 females with SVCAH experiencing 25 pregnancies, 13 carried to term. Nine of these 13 required cesarean sections because of pelvic disproportion. This is not surprising because of the constrictive anatomy that often is present postoperatively.*

Meyer-Bahlburg has expanded on these items and considered other factors in this article. Readers with interest in this topic are encouraged to read in full the articles by Meyer-Bahlburg abstracted in this issue of GGH *by myself and/or the article in* GGH *1999;15:3.*

Robert M. Blizzard, MD

X Inactivation: The Lyon Repeat Hypothesis

X chromosome inactivation is used by mammals to compensate for females having 2 X chromosomes while males have only 1. As established originally by Mary Lyon nearly 40 years ago, 1 of the 2 X chromosomes in females becomes transcriptionally inactive in every cell of the early embryo and remains so in somatic cells throughout life. This highly unusual form of gene regulation, in which almost a whole chromosome is silenced, has remained poorly understood until recently. Advances in the recent past include recognition that X chromosome inactivation starts at an X inactivation center and spreads for long distances to cover most of the X chromosome. Inactivation may spread to autosomes in instances of X:autosome translocation, although distances are shorter and inactivation is less efficient. A gene has been found (*Xist* in mice, *XIST* in humans) to reside at the inactivation center that is expressed only on the inactive X chromosome. It encodes a nontranslated RNA that after initiation of inactivation spreads to coat the entire inactive X chromosome and portions of autosomes in X:autosome translocations.

How *Xist* RNA spreads and how it silences genes has remained a mystery. It was suggested about a decade ago that "way stations" or "boosters" exist along the X chromosome that promote spreading. Lyon recently suggested that these boosters might be LINE-1 (L1) elements, for which there was evidence of their presence in higher abundance on the X chromosome than on autosomes in mice and humans. The term LINE refers to long interspersed repeat elements that are mammal-specific, autonomous mobile DNA sequences. According to a recent review by Kazazian and Moran, the human genome is comprised of roughly 15% L1 elements that over time have been inserted into the genome through reiterative rounds of reverse transcription. These events have expanded our genome in both size and complexity.

Bailey and colleagues have new evidence to support the role of L1s in X chromosome inactivation. They carefully examined DNA sequence data from GenBank from the human chromosomes X, 6, 7, 20, 21, and 22 for evidence of interspersed repeat elements. At the time of analysis, in late 1999, the X chromosome sequence was 34% complete. The other chromosomes, which served as autosomal controls, were 19%, 43%, 22%, 39%, and 69% complete, respectively. Using software that recognized repeat sequences, they identified 43% of total available human DNA sequence as interspersed repetitive sequence. It fell primarily into LINE, SINE (short interspersed repetitive element), LTR (long terminal repeat), and DNA repeat element categories of repetitive sequence. The X chromosome had a significantly higher con-

tent of interspersed repeats than other chromosomes, 52% vs 40%. More dramatic was the disparity of L1 elements, which accounted for most of the difference between the X chromosome and autosomes. The genome average for L1 elements was 16%, close to the 15% mentioned earlier. However, the L1 element content of the X chromosome was 27% compared with 13% for the autosomes. In other words, the abundance of non-L1 repetitive elements is comparable between the X chromosome and autosomes, but L1 elements are about twice as abundant on the X chromosome.

Further analysis revealed that the L1 elements cluster at Xq13, which in humans is where the X inactivation center and the *XIST* locus reside. Finally, they observed that the L1 content of Xp22, which contains genes that escape X inactivation, was lower than other regions of the X chromosome and similar to that of the autosomes.

Both Bailey et al and Lyon argue that these observations strongly support the possibility that the L1 elements serve as boosters to propagate the spreading of *Xist* RNA during X chromosome inactivation. One scenario suggests that clusters of L1 elements serve as binding sites for *Xist* or *Xist*/ protein complexes that promote packaging of dense (transcriptionally inactive) heterochromatin. Both concede the possibility that insertion of L1s is a consequence of rather than a causative factor for X chromosome inactivation.

The evolutionary aspect of these findings is very interesting. Bailey et al point out that there are subfamilies of L1 elements and that enrichment of L1 elements on the human X chromosome is from younger elements, in particular, those active 60 to 100 million years ago at the time when placental mammals diverged from marsupial mammals. The authors raise the possibility that accumulation of L1 elements was co-opted by placental mammals to construct an efficient X inactivation mechanism. If so, it would mean that repetitive material, often dismissed as junk, acquired a fundamental role in genetic regulation of the mammalian genome.

Bailey JA, et al. *Proc Natl Acad Sci USA* 2000;97:6634-6639.
Kazazian HH, Moran JV. *Nat Genet* 1998;19:19-24.
Lyon MF. *Proc Natl Acad Sci USA* 2000;97:6248-6249.

Editor's comment: *These papers and accompanying editorials nicely summarize the recent advances in understanding the mechanisms that contribute to X inactivation. The possibility that ancient mobile DNAs were co-opted during evolution to construct a complex mechanism to silence genes over long distances is fascinating. Those readers interested in the phenomena described here will be very interested in the following abstract.*

William A. Horton, MD

Phenotype Associated With a Ring (X) Relationship to XIST Locus

Small ring (X) chromosomes lacking the *XIST* gene at Xq13.2 have been associated with a severe phenotype that includes mental retardation, facial dysmorphism, and congenital abnormalities. It has been hypothesized that the loss of *XIST* results in functional disomy for the sequences contained in the ring. The investigators studied 47 females with a 45,X/46,r(X) karyotype and found 7 to have an *XIST*-negative ring. Only 1 of the 7 patients had the severe phenotype. The remaining 6 patients had physical phenotypes consistent with Turner syndrome. The rings were characterized cytogenetically and molecularly.

The severe phenotype in 1 patient can be explained by the absence of *XIST* expression, the relatively large amount of Xp material in the ring, and, possibly, the concomitant maternal uniparental isodisomy. The investigators propose 3 explanations for the unexpectedly mild phenotypes in the remaining 6 patients: (1) The rings contained limited amounts of X chromosome material, and sequences that when functionally disomic result in a severe phenotype were absent; (2) mosaicism resulted in the absence of the ring from tissues such as the brain that are important in the severe phenotype; and (3) an inactive X was present in some tissues at some time, as exemplified by the demonstration of *XIST* expression in 1 patient.

Please Send Correspondence to:
Robert M. Blizzard, MD
University of Virginia, The Blake Center
1224 West Main Street, 7th Floor, Suite 701
Charlottesville, VA 22903

Turner C, et al. *Hum Genet* 2000;106:93-100.

Editor's comment: *The presence of the severe phenotype in Turner syndrome was nicely explained previously by the possibility of functional disomy of some parts of the X chromosome. However, this report suggests the situation is more complicated and that each patient needs to be individually studied. The most likely explanation seems to be related to the amount of functional X chromosome DNA that is not inactivated. However, because every tissue in affected individuals is not usually studied, and since these tissues are not studied at various stages of development, all the answers are not in. In the past we have considered an exceptional patient as "weird." Now there seems to be an opportunity to answer many very basic questions. In the past, most of the reported patients were probably selected because of the severe phenotype. The actual mechanism producing the Turner phenotype may come to light by the study of such unusual patients. It is important to look for rings and evaluate these in relation to tissue locations and clinical phenotype.*

Judith G. Hall, OC, MD

Maternal Uniparental Disomy 7 (Syndrome): Review and Further Delineation of the Phenotype

Uniparental disomy (UPD) is defined as the inheritance of both homologous chromosomes from only 1 parent. So far, maternal UPD 7 has been described in 28 cases. Here, the authors report 4 new cases, present clinical information on 5 cases previously reported by the authors, and review the clinical and molecular findings of all 32 cases. The authors found a phenotype characterized by prenatal and postnatal growth retardation, occipitofrontal head circumference in the lower normal range, a triangular face, and retarded bone maturation. Findings of the facial gestalt included a high and broad forehead and a pointed chin. A broad mouth with downturned corners, prominent ears, café-au-lait spots, hemihypotrophy, or clinodactyly were rarely present. Psychomotor development was delayed in 6 cases. The clinical findings strikingly resemble the phenotype of the heterogeneous Russell-Silver syndrome (RSS). Other anomalies were found less frequently than in RSS. Molecular investigations revealed 11 cases with isodisomy and 17 cases with heterodisomy. In 4 cases this information was not available. From the allelic distribution of the microsatellites investigated, 9 cases might be the consequence of an error at maternal meiosis I, and 6 cases might be due to nondisjunction at maternal meiosis II. Three of the 17 heterodisomic cases had trisomy 7 in chorionic villi. In the remaining cases no prenatal diagnosis through chorionic villus sampling was reported.

Kotzot D, et al. *Eur J Pediatr Res* 2000;159:247-256.

Table
Frequency of Clinical Findings in Cases With Maternal UPD 7 and Russell-Silver Syndrome (RSS) According to Wollmann et al

	Frequency in Maternal UPD 7	Frequency in RSS
Birth		
Length (<−1 SD)	13/15 (87%)	99%
Weight (<−1 SD)	15/20 (75%)	94%
OFC (>−1 SD)	05/10 (50%)	64%
Last examination		
Height (<−1 SD)	21/22 (95%)	99%
Weight (<−1 SD)	15/15 (100%)	100%
OFC (>−1 SD)	12/16 (75%)	64%
Retarded bone age	10/10 (100%)	100%
Hemihypotrophy	06/06 (100%)	51%
Psychomotor retardation	06/18 (33%)	37%
Triangular face	16/16 (100%)	79%

OFC, occipitofrontal [head] circumference.

Reprinted with permission from Kotzot D, et al. *Eur J Pediatr* 2000;159:247-256.

Editor's comment: *Kotzot et al's paper emphasizes in the Table the clinical features of the maternal UPD 7 syndrome, which often has characteristics of RSS.*

The head circumference (OFC) of maternal UPD 7 individuals was around the 50th percentile for gestational age. Height and weight remained below the 3rd percentile whereas OFC usually adjusts to about the 10th percentile over time. It is not clear whether psychomotor retardation is seen as a regular feature of maternal UPD 7 since several cases had complicated pregnancies and/or deliveries that could have affected the intellect. Six of the 32 cases had hemihypotrophy.

Final adult height is not known for maternal UPD 7, although it appears that it is below the 3rd percentile. Clinodactyly is common, and a triangular face with a high forehead and pointed chin also are frequently seen. Precocious puberty, simian creases, teeth anomalies, and a squeaky voice were not seen in the maternal UPD 7 cases. The long-term prognosis is not yet known.

Mothers of isodisomic cases had an average age of 27.1 years, whereas the mean maternal age for heterodisomic cases was 37.1 years, suggesting those in the latter group are likely to be derived from cases of trisomy. Trisomy 7 is a common finding in chorionic villi sampling. Trisomy 7 prenatally diagnosed cases should be investigated for maternal UPD 7. Since most maternal UPD 7 cases probably derive from trisomy 7, the possibility of mosaicism explaining variation also must be considered.

RSS is a very common cause of intrauterine growth retardation. The heterogeneity that must exist under the RSS label is evident since approximately 10% of RSS cases appear to have maternal UDP 7 syndrome. All cases of suspected RSS need to be investigated for maternal UPD 7.

Judith G. Hall, OC, MD

Preliminary Study of Growth Hormone Therapy for Crohn's Disease

The results of treating 37 *adults* aged 20 to 55 years who had moderate to severe, active Crohn's disease with daily GH injections for at least 2 years were reported. A combination of radiologic and histologic criteria was used to confirm the diagnosis. In this double-blind, placebo-controlled study, patients were treated by their usual physicians and received other medications at their physicians' discretion. A loading dose of GH 5 mg/d SC was given the first week, followed by 1.5 mg/d for the remaining 16 weeks of study. The 18 subjects in the control group received an equal volume of diluent.

The primary endpoint of the study was improvement in the Crohn's Disease Activity Index, which monitors the severity of the disease based on 8 clinical variables:
- number of liquid or soft stools per day*
- severity of abdominal pain*

- general well-being*
- presence or absence of abdominal mass
- weight
- use of antidiarrhea drugs
- presence or absence of intestinal manifestations
- hematocrit.

Subjects were assessed at baseline, 1 to 2 weeks after initiation of the study, and monthly thereafter. Laboratory studies were extensive. All subjects were instructed to increase their protein intake by at least 2 g/kg/d, which was monitored with 3-day food diaries.

At 30 days, the subjects treated with GH had a significantly greater reduction in the Crohn's Disease Activity Index than the placebo group (P=.02), with further decreases during the next 3 months. The 3 variables that most significantly improved were those marked with an asterisk in the above list. The change in the Crohn's Disease Activity Index scores are seen in the Table. In addition, at the end of 4 months the subjects in the GH group reduced their other drug requirements by 56%, compared with a 4% increase in the placebo group. Insulin-like growth factor 1 increased significantly in the GH group, but no other significant differences were observed between the groups in any of the other biomedical studies measured. The most frequent side effect in the GH group was edema, which occurred in 10 of 19, patients and headache which occurred in 5 of 19. These symptoms occurred only during the first 2 weeks of the study. Two subjects in the GH group had tumors detected during the study (renal tumor, benign schwannoma), as did 1 subject in the placebo group (precancerous cells of the esophagus and a benign polyp of the stomach).

Slonim AE, et al. *N Engl J Med* 2000;342:1633-1637.

Editor's comment: *This is an intriguing and potentially very important study. As pointed out in an accompanying editorial by R. Balfour Sartor of the University of North Carolina, Chapel Hill, the article by Slonim et al is provocative. There are a number of clinical questions about the optimal dose of GH, the frequency of administration, and the length of therapy that need to be considered. Also, whether intestinal fibrosis with possible resultant intestinal strictures might occur is not known. The mechanism of action resulting in improvement also is not known.*

Obviously, additional studies in both adults and children are desirable. A review of the literature as of July 2000 reveals only 1 report (Henker J. Eur J Pediatr *1996;155:1066-1067) of children with Crohn's disease being studied with GH administration. Three adolescents possibly benefited from GH therapy. Studies such as these are difficult to do but, hopefully, are being pursued.*

William L. Clarke, MD

Table
Changes From Baseline in the Crohn's Disease Activity Index Scores During 4 Months of Treatment With Growth Hormone or Placebo*

	Placebo			Growth Hormone			*P* Value†
Month	No. of Patients	Score	Change From Baseline	No. of Patients	Score	Change From Baseline	
0 (Baseline)	15	206±126	—	19	287±134	—	
1	15	202±115	−5±76	19	186±107	−100±135	0.02
2	15	235±109	29±77	18	172±110	−116±139	0.001
3	15	204±140	−3±91	17	148±123	−139±159	0.006
4	15	187±163	−19±63	17	145±124	−143±144	0.004

*Plus-minus values are means ± SD. Only the 15 patients in the placebo group for whom follow-up data were available were included in the analysis. Higher scores on the Crohn's Disease Activity Index indicate more disease activity.

†*P* values are for the comparison of the changes in scores between the 2 groups.

Reprinted with permission from Slonim AE, et al. *N Engl J Med* 2000;342:1633-1637.

Risk of Persistent Growth Impairment After Alternate-Day Prednisone Treatment in Children With Cystic Fibrosis

Lai and coworkers report growth data on children with cystic fibrosis who, at 6 to 14 years of age, participated in a trial of alternate-day prednisone (1 to 2 mg/kg body weight) and were followed for approximately 6 to 7 years. Their growth data were obtained from the Cystic Fibrosis Patient Registry. Of the 224 subjects, 151 received prednisone and 73 received placebo. All had mild to moderate lung disease when the trial began. Four years after its initiation, the clinical trial was discontinued when it was determined that the side effects of prednisone outweighed its potential benefits.

Sixty-eight percent of the subjects who were reevaluated were 18 years of age or older. Results were reported 10 years after the trial began. Their Z scores for height declined during prednisone therapy but catch-up growth began 2 years after treatment was discontinued. The mean height for boys 18 years or older was 4 cm years less than that in the placebo group (or 13 percentile points). However, in girls the difference in height between the placebo and treatment groups was no longer present 2 to 3 years after the discontinuation of prednisone (Figure on next page).

The effect of alternate-day prednisone therapy varied depending on the age at which treatment was given. Specifically, boys who started prednisone every other day at 6 to 8 years of age had declines in height Z scores that last-

ed for 10 years. Boys beginning prednisone at 8 to 12 years had catch-up gains beginning about 2 years after stopping therapy. Boys who started prednisone during adolescence (12 to 14 years of age) maintained their baseline *Z* scores. When indices of pulmonary status were controlled for, the negative association between the use of prednisone and the *Z* score for height remained strong ($P<0.001$) in boys after prednisone was discontinued, and none of the 3 indices of pulmonary function correlated significantly with *Z* scores for height.

The authors point out that it is well known that long-term treatment with pharmacologic doses of prednisone correlates with significant reductions in final height. The differences between the sexes and the degrees of growth suppression seen in this study also have been seen in other studies, including those of children with asthma. They speculate that this may be due to the more pronounced deceleration of normal growth rate in boys prior to puberty, which might make them more susceptible to additional slowing of growth, or perhaps the higher secretion of GH in girls prepubertally. They *conclude* that the benefits of prednisone therapy in terms of pulmonary function are not prolonged once therapy is discontinued and do not outweigh the deleterious effect on final growth.

The authors summarized the results as follows: The growth impairment caused by prolonged alternate-day therapy with prednisone in prepubertal boys with CF persisted posttreatment and significantly reduced adult height. Although children gained substantial weight with treatment, this weight did not persist posttherapy. Because of these findings and the failure of therapy to benefit CF symptoms long term, one must conclude that *prolonged therapy is not beneficial* in CF children. If used in the treatment of any disease, glucocorticoid therapy must be monitored and individualized carefully to achieve the lowest effective dose and the shortest duration of therapy possible in order to minimize the risk of permanent growth impairment, particularly in boys.

Lai H, et al. *N Engl J Med* 2000;342:851-888.

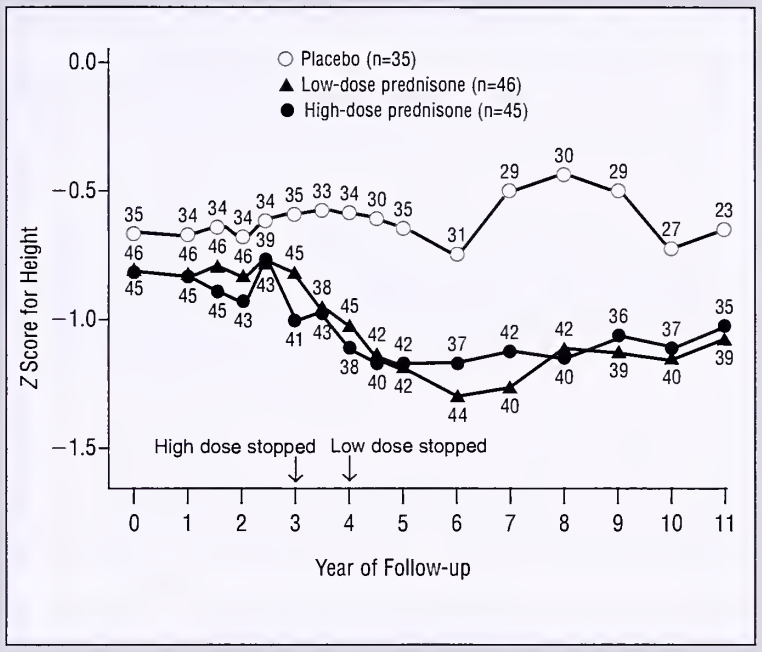
Editor's comment: *This important study demonstrates the significant negative impact of glucocorticoids on linear growth even when prescribed only every other day. It also is important because it stratifies and analyzes the response with regard to different ages at the onset of treatment. Of utmost importance, treatment caused diabetes and cataracts at a rate sufficiently high enough to warrant stopping this trial in 1991, 4 years after its initiation. Thus, the effects of glucocorticoids on other systems is potentially more significant medically than just its effect on height.*

Pamela Davis and Carolyn Kercsmar from Cleveland have an excellent editorial in the same issue of the New England Journal of Medicine *(2000;342:887-888), entitled "Growth in Children With Chronic Lung Disease." Readers are encouraged to review the thoughtful and useful comments pertaining to the causes of growth retardation in CF and the alternatives for therapy. The authors emphasize that there are multiple reasons for growth failure in CF patients, that glucocorticoids have multiple toxic effects beyond those reported in the article by Lai et al, and that ibuprofen is a less toxic and more proficient anti-inflammatory agent than glucocorticoids. They add that there is a dearth of evidence concerning the efficacy or adverse effects of inhaled glucocorticoids, although 12% of patients with CF in the United States are treated with these. Davis and Kercsmar recommend that with the increased risk of diabetes, cataracts, osteoporosis, and the reduction in height, the price may be too high to pay to use glucocorticoids in CF, especially since the benefits of anti-inflammatory therapy can be achieved in other ways in children with this disease.*

The attention of readers interested in this topic is called to the lead article in GGH *(2000;16[2]:21-26) written by Drs. O. Mehls and B. Tönshoff of Heidelberg. The title is "Effects of Glucocorticoids on Growth."*

William L. Clarke, MD

Figure
Relation of Z Scores for Height to Years of Follow-up in Boys With Cystic Fibrosis Who Received Placebo, Low-Dose Prednisone, or High-Dose Prednisone



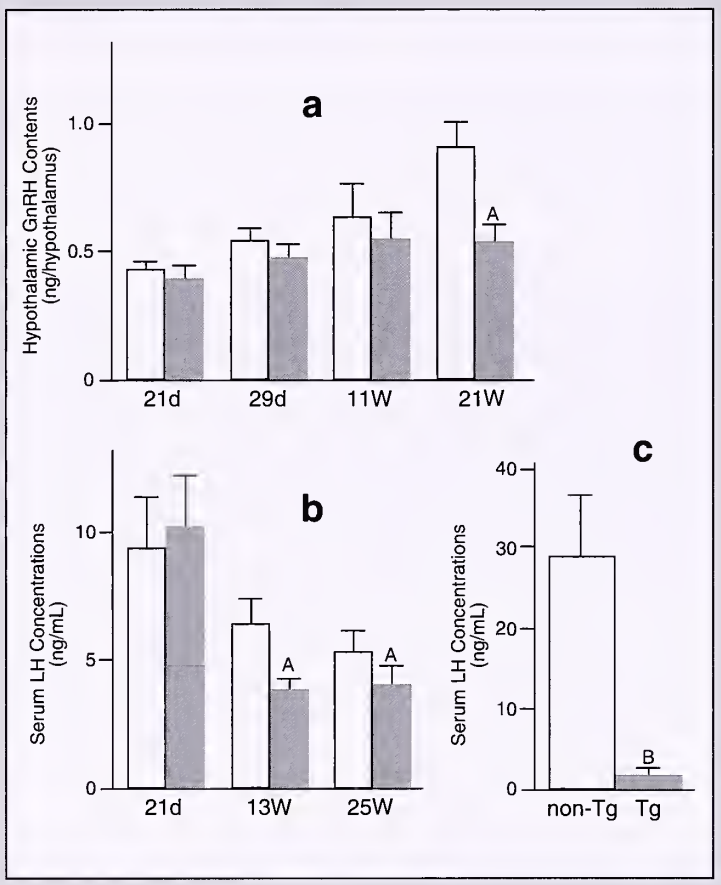
The low dose of prednisone was 1 mg/kg, and the high dose was 2 mg/kg. The number of subjects at each point of follow-up is indicated. Among the boys, *Z* scores for height remained significantly lower after 10 years in those who received prednisone than in those who received placebo ($P=0.03$). A *Z* score of zero corresponds to the 50th percentile of the reference population. A *Z* score of −1.0 indicates 1 SD below the mean, which corresponds approximately to the 15th percentile.

Reprinted with permission from Lai H, et al. *N Engl J Med* 2000;342:851-888.

Accelerated Puberty and Late-Onset Hypothalamic Hypogonadism in Female Transgenic Skinny Mice Over-Expressing Leptin

Transgenic skinny mice were generated by causing overexpression of leptin under the regulation of a liver-specific promoter (human serum amyloid P component). In these animals there is chronic hyperleptinemia (81 ng/mL) compared with nontransgenic litter mates (NTLM; 9 ng/mL). Hypophagia is present, white and brown adipose tissue disappears, and insulin sensitivity and glucose metabolism increase (Ogawa Y, et al. *Diabetes* 1999;48:1822-1829).

Figure



Hormonal profile of transgenic (Tg) skinny mice overexpressing leptin (filled columns) and their nontransgenic (non-Tg) littermates (open columns). (*a*) Hypothalamic gonadotropin hormone-releasing hormone (GnRH) contents. (*b*) Serum luteinizing hormone (LH) concentrations 15 minutes after intraperitoneal administrations of GnRH. Procedures were performed on day 21 (21d) (filled columns, $n = 10$; open columns, $n = 8$; on the diestrus day at 13 weeks (13W) (filled columns, $n = 6$; open colunms, $n = 4$; and on the diestrus day at 25 weeks (25W) (filled and open columns, $n = 10$) of age. (*c*) Serum LH concentrations at 2,000 hours on the proestrus day between 13 and 18 weeks of age (filled columns, $n = 6$; open columns, $n = 4$). $^{A}P < 0.05$ compared with nontransgenic littermates by ANOVA with Fisher's least significance difference test. $^{B}P < 0.005$ by Student's test.

Reprinted with permission from Yura S, et al. *J Clin Invest* 2000;105:749-754.

In the present study by Yura et al, heterozygous males and females with 30 copies of the leptin transgene were mated. *In the female offspring* generated for this study, vaginal opening occurred earlier in the transgenic skinny mice (27.3 vs 29.4 days) than in NTLM ($P<0.05$). The transgenic animals had larger ovarian follicles but comparable ovarian weights. Uterine weights were significantly increased (22.3 g vs 13.3 g in NTLM; $P<0.005$). The skinny and NTLM females were comparably fertile at 8 weeks, *but not at 22 weeks*. At that time, the skinny animals were markedly subfertile with markedly reduced ovarian weights, follicular atrophy, decreased basal and gonadotropin hormone-releasing hormone (GnRH)–stimulated serum luteinizing hormone concentrations, and reduced hypothalamic GnRH values compared with NTLM. Gonadotropin administration restored ovarian size and morphology to those of NTLM. In contrast, *in males* there was no significant difference in fertility, testicular weights or morphology, or hypothalamic GnRH content between transgenic mice and NTLM.

The investigators concluded that transgenic *female mice* with hyperleptinemia undergo earlier pubertal maturation than do NTLM and have comparable fertility at younger ages despite no apparent adipose tissue; when older, however, they develop hypogonadotropism due to decreased GnRH production. The mechanism of the latter effects was attributed to downregulation of "hypothalamic leptin signaling." They suggest that the hypothalamic effects of leptin on feeding and reproduction traverse separate and distinct pathways, and that there also is a gender difference in leptin responsiveness.

Yura S, et al. *J Clin Invest* 2000;105:749-754.

Editor's comment: *According to the "critical weight" hypothesis and clinical experience, body fat is extremely important in promoting normal linear growth and sexual maturation in both males and females, but particularly in females. These investigators have developed an animal model in which sexual maturation is normal/accelerated in males and females but that cannot be maintained in older females, probably due to a decrease in hypothalamic GnRH production. The data confirm the significant role that leptin plays in the regulation of the early maturation of the reproductive endocrine system. The data also complement previous studies in which leptin has been administered to normal or leptin-deficient (but responsive) animals. One wonders if the hyperleptinemia of the obese teenage male may sometimes paradoxically delay the onset of puberty. On the other hand, the hypothalamic hypogonadism that may occur in some obese adult women, but seldom in obese adult males, also may reflect an effect of chronic hyperleptinemia.*

Allen W. Root, MD

Long-Term Outcome of Classical 21-Hydroxylase Deficiency: Diagnosis, Complications and Quality of Life

A nationwide search for patients with classic 21-hydroxylase deficiency (21-OHD) was undertaken in Finland to determine the long-term outcome of the disease. One hundred and eight patients were found. Fifty-four (31 females, 23 males), or 50%, had the salt-wasting form of congenital adrenal hyperplasia (CAH). Another 54 (29 females, 25 males), or 50%, had the simple virilizing form from 21-OHD. The age at diagnosis was delayed in males compared with females (Figure). A significant number of severe complications suggestive of glucocorticoid deficiency was found. There were 5 deaths possibly connected with cortisol deficiency (4.6% of all patients). Ten additional patients (9.3%) had been acutely admitted to the hospital 14 times due to symptoms of glucocorticoid deficiency. These symptoms included sudden loss of consciousness, convulsions, and severe fatigue. Afterwards, permanent neurologic defects were detected in 2 of these patients.

Finally, a cross-sectional study was carried out to establish an estimate of the long-term outcome of the disease. Thirty-two, or 55%, of the 58 patients ≥16 years of age participated in this study. The patient group did not differ from the general Finnish population in terms of education. Three of the patients (5%) had retired prematurely. Surprisingly, the patients felt that their health-related quality of life, as reported in the RAND-36 questionnaire, was better than that of the general Finnish population (P=0.023). However, since a significant number of all qualifying patients did not participate in this study, the quality-of-life evaluation results must be interpreted with caution.

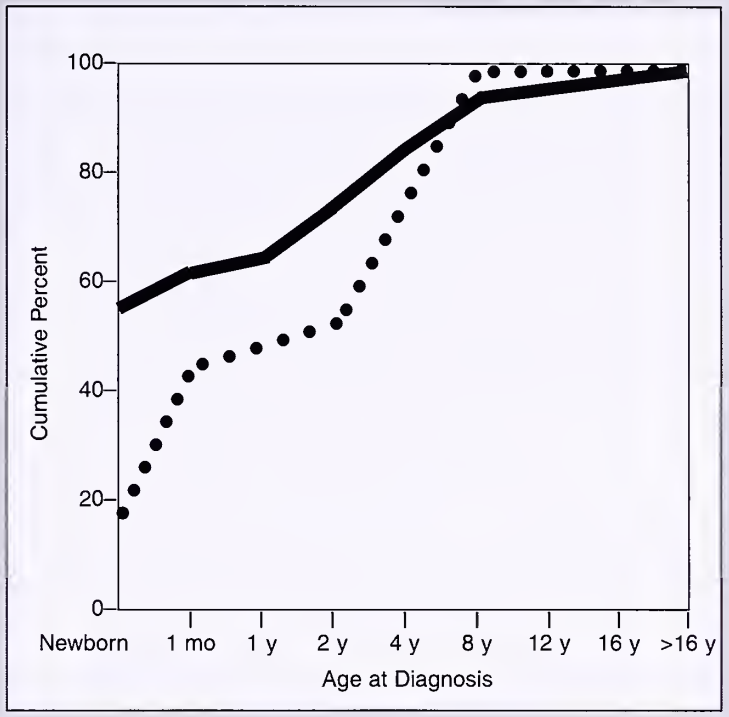
The authors conclude that a significant number of complications was found among patients treated for classic 21-OHD. Nevertheless, the disease has a favorable outcome in terms of quality of life.

Jääskeläinen J, Voutilainen R. *Acta Paediatr* 2000;89:183-187.

Editor's comment: *This report complements the recently published review of the long-term consequences of CAH by Blizzard in* GGH *(2000;15[3]:33-41) and detailed appraisal of the pathophysiology of this disorder by White and Speiser (*Endocr Rev *2000;21[3]:245-291). The larger number of females than males with classic CAH is consistent with the probabilities that some males were not identified and that others perished without diagnosis. Conspicuously absent in this report are clear data on independence and interpersonal relationships, marriage, parenting, and other intimate details that bear on the reproductive function of adults with CAH. However, it is encouraging to learn that the general quality of life related to health in many adults with CAH is good; this may reflect the greater patient-physician contact required by those with CAH, although this point was not investigated. The fact that patients with CAH still die of relatively minor illnesses or become acutely ill because of insufficient glucocorticoid replacement is a sober reminder of this hazard.*

Allen W. Root, MD

Figure



Cumulative percentage of females (continuous line) and males (broken line) with classic 21-OHD in relation to age at diagnosis.

Reprinted with permission from Jääskeläinen J, Voutilainen R. *Acta Paediatr* 2000;89:183-187.

A Study of Chromosome Aberrations After rhGH Treatment

Because of the suggestion that rhGH therapy might be associated with certain forms of leukemia, Slyper et al undertook to evaluate patients before and after rhGH therapy. They also looked for carriers of conditions that might increase the levels of spontaneous and induced chromosomal aberrations, and thereby potentially increase susceptibility to neoplasm when treatment with GH is used.

The data collected are not exactly comparable to previous studies. In this study children with the conditions ordinarily treated with rhGH were studied. Metaphase cells were examined for spontaneous chromosomal and chromatid aberrations before and after 6 months of treatment with rhGH. In addition, cells from these individuals were exposed to radiation to assess chromosome fragility. Dicentric and reciprocal translocations were specifically sought. They excluded patients with preexisting malignancy, those who had previous radiotherapy or chemotherapy, and those with syndromes that were known to be at risk for malignancy. The investigators studied only metaphase cells that were at first mitotic division after mitogenic stimulation. Chromatid-type aberrations in which there were deletions or exchanges such as triradials and quadraradials were sought. Five hundred cells were

examined from each individual for spontaneous aberrations. Two hundred cells from each person were examined for radiation-induced aberrations. In order to be certain that cells were examined at first mitotic division, the investigators used 5-bromo-2′-deoxyuridine (BrdUrd) and phytohemagglutinin (PHA).

No patient showed a significant increase in aberrant cells with treatment. However, the mean frequency of chromatid-type aberrations was significantly higher after treatment on a per cell basis. Because 2 patients contributed inordinately to this increase, they repeated the studies on these 2 patients. No remarkable changes occurred with time. There also was a low frequency of ring chromosomes in the 6-month samples.

Although these data are not totally comparable to other studies, no real cause for concern about the risk that GH therapy predisposes to leukemia was generated.

Slyper AH, et al. *Pediatr Res* 2000;47:634-639.

Editor's comment: *Clearly, if there is a risk from GH therapy, it needs to be identified so that it can be weighed against the benefits. The present study does not seem to suggest that there is a major risk. However, it suggests there may be a subpopulation of individuals who would be at risk or contribute to any increase in chromosomal aberrations. The unusual increase in observed leukemia in the Japanese population receiving rhGH certainly deserves further evaluation, and whether there are some subgroups receiving rhGH who might be at risk must be further evaluated.*

*Your attention is called to a previous paper by Dr. Slyper entitled "How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?" (*GGH *1998;14[1]:4-7) Dr. Slyper and his colleagues are providing recommendations and much-needed data to utilize in our considerations of rhGH as a therapeutic tool.*

Judith G. Hall, OC, MD

Inhaled Corticosteroid Use and Bone Mineral Density in Patients With Asthma

The investigators report the results of a cross-sectional survey of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) in a basically healthy, young adult population (20 to 40 years of age; females 119, males 77) who because of mild asthma had received inhaled glucocorticoids (primarily beclomethasone, median dose=876 mg; few to no doses of oral, parenteral, or dermal preparations) for a median period of 6 years. An inverse relationship between the *cumulative* dose of glucocorticoids and the BMD of the lumbar spine (L2-L4), left femoral neck, trochanter, and Ward's triangle was found. No relationship was found between the *daily* dose of glucocorticoids and BMD at any site, nor did any subject have a vertebral fracture. Although mean BMD measurements were normal at all sites, doubling of the *cumulative* dose of inhaled agents resulted in a "decline" in BMD of approximately −0.03 SD at all sites (approximately −0.020 g/cm^2 at L2-L4). The *total duration* of inhaled corticosteroid intake also was inversely related to BMD at each site. The authors estimated that if a patient received a *cumulative* dose of 5,100 mg of inhaled corticosteroids over 7 years, the L2-L4 BMD would fall 1 SD; if continued over longer periods, the patient could be at substantial risk for osteopenia and fracture.

Wong CA, et al. *Lancet* 2000;355:1399-1403.

Editor's comment: *Although these data were accumulated in young adults, they have clear implications for children, many of whom receive prolonged courses of inhaled glucocorticoids for treatment of asthma. Inhaled glucocorticoids have been associated with impairment of growth and adrenal function in children.[1] Glucocorticoids adversely affect chondrocyte proliferation and skeletal mineralization; they depress bone formation by suppressing osteoblastogenesis and hastening osteoblast apoptosis, enhance bone resorption, decrease intestinal absorption of calcium, and increase urinary excretion of calcium.[2,3] Records quantitating the cumulative dose of inhaled glucocorticoids should be maintained on all subjects receiving them. It has been suggested that BMD be determined in the young adult after he/she has received 5,000 mg of these agents and consideration be given to administration of a bisphosphonate in order to prevent glucocorticoid-induced bone loss.[4] Careful study of mineral metabolism and BMD in children receiving these medications is warranted and necessary. The readers may be interested in the lead article in* GGH *(2000;16[2]:21-26) entitled "Effects of Glucocorticosteroids on Growth," by Drs. O. Mehls and B. Tönshoff of Heidelberg.*

Allen W. Root, MD

1. Allen DB. *Acta Paediatr* 1998;87:123-129.
2. Manelli F, Giustina A. *Trends Endocrinol Metab* 2000;11:79-85.
3. Manolagas SC, Weinstein RS. *J Bone Miner Res* 1999;14:1061-1066.
4. Sambrook PN. *Lancet* 2000;355:1385.

GROWTH, Genetics, & Hormones is published under educational grants from Genentech, Inc. and Schwarz Pharma AG. The information reflects the views of the editors and/or contributors and not necessarily those of the sponsor, grantors, or the publisher.

Published by:

Gardiner-Caldwell SynerMed

405 Trimmer Road
PO Box 458
Califon, NJ 07830

Intrauterine Growth Retardation Associated With Maternal Uniparental Disomy for Chromosome 6 Unmasked by Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is caused by steroid 21-hydroxylase deficiency. The gene (*CYP21*) for this enzyme is located on the short arm of chromosome 6 (6p21.3).

This enzyme deficiency leads to reduced conversion of 17-hydroxy progesterone to 11-deoxycortisol, resulting in a deficiency of cortisol and overproduction of androgens. In female newborns this disorder is associated with ambiguous genitalia. Untreated children show rapid growth, phallic enlargement, precocious pubarche, early epiphyseal closure, and short stature.

In this report, 1 female newborn with intrauterine growth retardation (IUGR) and CAH was found to be homozygous for a rare exon 4 mutation 1172N. The patient showed transient delayed mental development, evidence of early puberty, increased bone age, and accelerated growth. Genetic analysis found that only the mother was heterozygous for this mutation. Further DNA microsatellite analysis confirmed the diagnosis of uniparental disomy.

Spiro RP, et al. *Pediatr Res* 1999;46:510-513.

Editor's comment: *Uniparental disomy is a condition in which both copies of a chromosome segment are inherited from a single parent. There is only 1 other report of a patient with uniparental disomy of the same segment of 6p (van den Berg-Loonen EM, et al. Hum Immunol 1996;45:46-51). This patient had IUGR at birth. Clinical symptoms appear to be due to genetic imprinting or expression of recessive traits from the affected chromosome segment and not directly associated with uniparental disomy. Similar reports of uniparental disomy involving the long arm of chromosome 6 have been associated with neonatal diabetes but not IUGR. These data are suggestive that fetal growth gene(s) are located on the short arm of chromosome 6 and that genetic mutations in this particular area (6p21.3) will cause IUGR. This case study shows that a rare underlying genetic mutation can cause multiple clinical manifestations. However, the risk of recurrence of these mutations is negligible in families.*

Fima Lifshitz, MD

GROWTH, Genetics, & Hormones Table of Contents for Volume 16, 2000

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Follow the instructions listed there to receive CME Category 1 credit. Please note that each question may have more than one correct answer.

1. MODY differs from type 1 diabetes in which of the following ways?
   a. having an autosomal dominant inheritance pattern
   b. by not requiring insulin for at least 5 years after the onset of DM
   c. having no complications in later life
   d. usually responds initially to oral hypoglycemic agents

2. MODY currently is believed to be attributable by most authorities to:
   a. insulin resistance
   b. growth hormone excess
   c. an insulin secretory defect

3. Currently:
   a. there are 5 types of MODY
   b. there are 3 types of MODY associated with severe diabetes
   c. low birth weight is thought to occur in MODY type 2
   d. renal dysfunction is often found in MODY type 5
   e. the data are persuasive that MODY type 1 is similar to type 2 diabetes in respect to the occurrence of diabetic complications

4. Which of the following qualify the issuance of a patent on a biological material?
   a. the product must be new, useful, and nonobvious
   b. a product of nature converted to a new form; eg, a purified or synthetic DNA compound, a product coupled to a non-native promoter, or a product inserted in a vector

Answer Key: 1. a, b, d 2. c 3. a, b, c, d, e 4. a, b

CME Accreditation Statement

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

UNIVERSITY OF VIRGINIA HEALTH SCIENCES CENTER
SCHOOL OF MEDICINE

The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Disclosure: As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Stoffers, Noonan, Clarke, Hall, Horton, and Lifshitz report no conflicts. Dr. Root serves on Genentech Inc.'s National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

GROWTH, Genetics, & Hormones is published under unrestricted educational grants from Genentech, Inc. and Schwarz Pharma AG.

Robert M. Blizzard, MD
c/o Gardiner-Caldwell SynerMed
405 Trimmer Road
PO Box 458
Califon, NJ 07830